Effects of Ocean Acidification on Early Life History Stages of Caribbean Scleractinian Corals

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EFFECTS OF OCEAN ACIDIFICATION ON EARLY LIFE HISTORY STAGES OF CARIBBEAN SCLERACTINIAN CORALS

By

Rebecca Albright

A DISSERTATION

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EFFECTS OF OCEAN ACIDIFICATION ON EARLY LIFE HISTORY STAGES OF CARIBBEAN SCLERACTINIAN CORALS

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Ocean acidification (OA) refers to the increase in acidity (decrease in pH) of the ocean’s surface waters resulting from oceanic uptake of atmospheric carbon dioxide (CO₂). Mounting experimental evidence suggests that OA threatens numerous marine organisms, including reef-building corals; however, few studies have focused on the effects on early life history stages. Coral recruitment is critical to the persistence and resilience of coral reefs and is regulated by several early life processes, including: larval availability (gamete production, fertilization, etc.), larval settlement, post-settlement growth, and survival. Environmental factors that disrupt these early life processes can result in compromised or failed recruitment and profoundly affect future population dynamics. To evaluate the effects of OA on the sexual recruitment of corals, sexual reproduction (including fertilization and sperm swimming speeds) and several critical early life history stages (including larval metabolism, larval settlement, and post-settlement growth) were tested in common Caribbean coral species. Three pCO₂ levels were used: ambient seawater (380 μatm) and two pCO₂ scenarios that are projected to occur by the middle (560 μatm) and end (800 μatm) of the century as determined by the Intergovermental Panel on Climate Change. Results show that fertilization success, larval metabolic rates, larval settlement rates, and post-settlement growth rates are all compromised with increasing pCO₂. This dissertation demonstrates that OA has the
potential to negatively impact sexual reproduction and multiple early life history processes of several common Caribbean coral species and may contribute to substantial declines in sexual recruitment that are felt at the community and/or ecosystem scale.
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Chapter 1: Coral Reef Ecosystems and Ocean Acidification

Introduction

Coral reefs cover an estimated 284,300 to 600,000 km^2 of the world’s tropical and subtropical oceans (Smith 1978; Spalding et al. 2001), providing a net economic benefit of approximately US$30 billion per year (Cesar et al. 2003) and harboring one of the most diverse ecosystems on the planet in terms of species complexity (Veron 1995). Despite the profound ecological and economic importance of these valuable marine ecosystems, global degradation of coral reefs has resulted in unprecedented loss over the past several decades (Hoegh-Guldberg 1999; Hughes et al. 2003). One-fifth of the world’s coral reefs have effectively been destroyed and show no immediate signs of recovery (Wilkinson 2008). Caribbean waters have been particularly devastated; a study released in 2003 estimated an 80% reduction in relative coral cover (50% to 10% in absolute terms) over the last thirty years (Gardner et al. 2003). Two years later, the warmest year in >150 years resulted in the most severe and extensive mass coral bleaching event ever recorded in the Caribbean, with bleaching observed in over 80% of corals and mortality in over 40% at many sites (Eakin et al. 2010). Of the remaining global reefs, approximately 15% are under imminent threat of collapse (within the next 10-20 years), and an additional 20% face long-term demise (loss in 20-40 years). Scleractinian (reef-building) corals act as ecosystem engineers in coral reef ecosystems, structuring the habitat and driving key ecological processes such as marine invertebrate and fish recruitment. Carpenter et al. (2008) characterized ~33% of 704 reef-building coral species as having an elevated risk of extinction and suggested that most of these species could become extinct by 2100 given current trends in climate change.
Given the current trends in reef destruction, it is paramount to understand processes that may allow these valuable marine ecosystems to persist. The persistence and recovery of a population, and of a species, requires that levels of recruitment keep pace with the loss of adult individuals (Richmond 1997; Hughes & Tanner 2000). Although corals can reproduce asexually, sexual reproduction plays a critical role in maintaining genetic diversity, (re-)populating denuded areas, and facilitating the recovery of reefs post disturbance (reef resilience). Successful coral recruitment, defined here as the introduction and successful inclusion of a new individual to a population, is determined by three sequential life history stages: (1) larval availability (including gamete production and successful fertilization), (2) settlement ecology (related to larval and substrate condition), and (3) post-settlement ecology (growth and survival of the newly settled individual) (Figure 1.1) (Ritson-Williams et al. 2009). Larval settlement (when planulae first attach to the benthos) and subsequent survival (recruitment) are processes that can control marine population dynamics (Gaines & Roughgarden, 1985; Doherty & Fowler, 1994); environmental factors that disrupt these various processes can result in compromised recruitment or recruitment failure and profoundly affect marine population dynamics (Gaines & Roughgarden 1985; Harrison & Wallace, 1990; Doherty & Fowler, 1994; Riegl et al. 2009).

Recruitment is influenced by both pre-settlement and post-settlement processes. Environmental factors known to influence planktonic development and survivorship, as well as various aspects of settlement include nutrients (Harrison & Ward 2001; Bassim et al. 2002), anthropogenic contaminants (Negri & Heyward 2001; Negri et al. 2005), salinity (Vermeij et al. 2006), temperature (Edmuns et al. 2001), and exposure to ultra-
Figure 1.1. Successful coral recruitment is determined by three sequential life history stages: (1) larval availability (including gamete production and successful fertilization), (2) settlement ecology (related to larval and substrate condition), and (3) post-settlement ecology (growth and survival) (adapted from Ritson-Williams et al. 2009, redrawn with permission from M. Vermeij).

violet radiation (UVR) (Wellington & Fitt 2003; Gleason et al. 2006). Post-settlement survivorship has been shown to be influenced by habitat choice (Miller et al. 2000; Harrington et al. 2004); water quality (Koop et al. 2001; Villanueva et al. 2006); sedimentation (Hunte & Wittenberg 1992; Babcock & Smith 2002); and indirect (allelopathy, Kuffner & Paul 2004; shading, Box & Mumby 2007) and direct competition with algae (Box & Mumby 2007) and other sessile organisms. A detailed review of ecological factors affecting coral recruitment is provided by Ritson-Williams et al. (2009). Despite recent efforts to constrain relationships between a variety of
environmental factors and early life history stages of corals, few studies have investigated the effects of increasing levels of ocean acidification. The pH of the ocean’s surface waters has already declined by 0.1 pH units since the industrial revolution (~year 1880) due to anthropogenic addition of CO$_2$ to the atmosphere. This represents a 30% increase in acidity (IPCC 2007). Due to the changes in seawater chemistry that are already taking place, a better understanding of how changing water chemistry influences coral recruitment is fundamental to coral reef ecology and management.

**Ocean Acidification and Carbonate Chemistry**

Ocean acidification (OA) refers to the increase in acidity (decrease in pH) of the ocean’s surface waters resulting from oceanic uptake of atmospheric CO$_2$. Present-day atmospheric carbon dioxide ($p$CO$_2$) levels are estimated to be 387 ppm (IPCC 2007), 30% higher than the natural range over the last 650,000 years (Figure 1.2) (Siegenthaler et al. 2005). $p$CO$_2$ levels are increasing at a rate of 0.5% per year (IPCC 2007), 200 times faster than any changes that occurred during the last eight glacial cycles (Siegenthaler et al. 2005) and 8-15 times faster than any changes in the past 60 Myr, including the Paleo-Eocene Thermal Maximum (PETM) (Zeebe et al. 2009). $p$CO$_2$ levels are projected to double present day values (~750 ppm) by the end of this century (year 2100; IPCC 2007) (Figure 1.3).

Increasing $p$CO$_2$ leads to a rise in the CO$_2$ concentration of the ocean’s surface waters via gas exchange at the air sea interface (Takahashi et al. 1997). Approximately one-third of all CO$_2$ emissions from the past 200 years have been absorbed by the oceans (Sabine et al. 2004). On dissolution in seawater, CO$_2$ reacts with H$_2$O, triggering a series of chemical reactions that alter the seawater carbonate chemistry. The basic equation
Figure 1.2. (a) Historical $pCO_2$ concentrations derived from EPICA and Vostok ice cores. The orange bar at the right indicates 50 yrs of Mauna Loa measurements. Colored arrows correspond to the 6 illustrative SRES scenarios (Figure 1.3) and their projected $pCO_2$ values for the year 2100 (IPCC 2007). (b) Monthly mean atmospheric carbon dioxide at Mauna Loa Observatory, Hawaii. The $CO_2$ data constitutes the longest record of direct measurements of $CO_2$ in the atmosphere. The red line represents the monthly mean values. The black curve represents seasonally corrected data. (Dr. Pieter Tans, NOAA/ESRL (www.esrl.noaa.gov/gmd/ccgg/trends/)).

Describing the carbonate equilibrium system are:

\[
(CO_2)_{aq} + H_2O \leftrightarrow H_2CO_3 \quad (1)
\]

\[
H_2CO_3 \leftrightarrow H^+ + HCO_3^- \quad (2)
\]

\[
HCO_3^- \leftrightarrow H^+ + CO_3^{2-} \quad (3)
\]
where \( \text{CO}_2 \) = carbon dioxide, \( \text{H}_2\text{CO}_3 \) = carbonic acid, \( \text{HCO}_3^- \) = bicarbonate ion, and \( \text{CO}_3^{2-} \) = carbonate ion. The net chemical reaction that occurs when \( \text{CO}_2 \) reacts with seawater is:

\[
\text{H}_2\text{O} + (\text{CO}_2)_{aq} + \text{CO}_3^{2-} \rightarrow 2\text{HCO}_3^- \quad (4)
\]

In result, \([\text{CO}_2]_{aq}\) and \([\text{HCO}_3^-]\) increase, while \([\text{CO}_3^{2-}]\), pH, and the carbonate saturation state (\(\Omega\)) decrease, causing surface waters to become more acidic (Figure 1.4) (Broecker et al. 1979; Caldeira & Wickett 2003; Sabine et al. 2004). Increasing atmospheric \(\text{CO}_2\) concentrations have already depleted seawater carbonate concentrations by \(\sim 30\ \mu\text{mol kg}^{-1}\), simultaneously reducing the pH of the ocean’s surface waters by 0.1 units relative to the preindustrial value of 8.18 (a 30% increase in [\(\text{H}^+\)]) (IPCC 2007). Further reductions of 0.3–0.5 pH units are projected by the end of this century as the oceans continue to absorb anthropogenic \(\text{CO}_2\) (Sabine et al. 2004; IPCC 2007).

Reductions in \(\text{CO}_3^{2-}\) lower the calcium carbonate saturation of the surface ocean, defined as:

\[
\Omega = [\text{Ca}^{2+}] [\text{CO}_3^{2-}] / K'_{sp}
\]

where \(K'_{sp}\) is the solubility product for a particular mineral phase of \(\text{CaCO}_3\) (e.g., aragonite, calcite). Throughout the remainder of this dissertation, saturation state refers to aragonite (\(\Omega_{\text{arag}}\)), the principal crystal form of \(\text{CaCO}_3\) in reef-building corals. \(\Omega_{\text{arag}} > 1\) indicates supersaturation with respect to aragonite and a greater ease of precipitation, while \(\Omega_{\text{arag}} < 1\) indicates undersaturation and a tendency towards dissolution. The surface waters of the tropical oceans are currently supersaturated (\(\Omega_{\text{arag}} = 4.0 \pm 0.2\), mean \(\pm SD\)) with respect to aragonite; however, \(\Omega_{\text{arag}}\) has steadily declined from a calculated \(4.6 \pm 0.2\) 100 years ago and is expected to continue declining, to \(3.1 \pm 0.2\) by the year 2065 and \(2.8 \pm 0.2\) by 2100 (Kleypas et al. 1999).
Figure 1.3. Changes in global average surface pH and saturation state with respect to aragonite in the Southern Ocean under various SRES scenarios. Time series of (a) atmospheric CO$_2$ for the 6 illustrative SRES scenarios, (b) projected global average surface pH and (c) projected average saturation state (with respect to aragonite) in the Southern Ocean. The A1 scenario family describes a future world characterized by rapid economic growth, low population growth, and rapid introduction of new, more efficient technologies. A2 scenarios are characterized by high population growth with economic and technological changes that are fragmented and slower than other scenario families. B1 scenarios have low population growth (similar to A1), but with rapid changes in economy and technology including the introduction of clean and resource-efficient technologies that emphasize environmental sustainability. B2 scenarios describe moderate population growth, intermediate levels of economic development, and less rapid technological change than A1 and B1 scenarios (IPCC 2007).
This reduction in saturation state will likely cause a global reduction in the rates of reef accretion, as the deposition of CaCO₃ by scleractinian corals and other reef organisms is partially controlled by $\Omega_{arag}$ (Figure 1.5) (Gattuso et al. 1998; Langdon et al. 2000, 2003; Leclercq et al. 2000, 2002; Marubini et al. 2001, 2002; Reynaud et al. 2003; Langdon & Atkinson 2005; Fine & Tchernov 2007). The rate of calcification of adult scleractinians and reef communities decreases by 11-40% with conditions mimicking a doubling of atmospheric CO₂ levels (Gattuso et al. 1998; Kleypas et al. 1999; Langdon et al. 2000; Leclercq et al. 2000; Marubini et al. 2001). These findings have led to speculation as to whether corals are approaching a critical threshold, beyond which their ability to calcify quickly enough to effectively form reefs will be severely compromised.

**Effect of Ocean Acidification on Early Life History Stages of Marine Invertebrates**

Ocean acidification is expected to have negative effects on a variety of marine organisms (Royal Society 2005), and early life history stages of these organisms may be
Figure 1.5. Effect of $\Omega_{\text{arag}}$ on calcification rate expressed as a percentage of the preindustrial rate ($\Omega_{\text{arag}}=4.6$). Data are from published studies on corals or coral reef communities (Langdon & Atkinson 2005).

more sensitive than adults, as has been demonstrated in oysters and echinoderms (reviewed by Kurihara 2008). The number of studies devoted to the potential impacts on early life history stages of marine invertebrates has risen over the past several years. Mounting experimental evidence now suggests that numerous biological and physiological processes will be negatively impacted as the oceans continue to acidify: sperm motility in urchins (Havenhand et al. 2008), corals and sea cucumbers (Morita et al. 2009); fertilization success in sea urchins (Kurihara & Shirayama 2004; Havenhand et al. 2008; Reuter et al. 2010; but see Byrne et al. 2010), molluscs (Parker et al. 2009, 2010; but see Havenhand & Schlegel 2009) and corals (Albright et al. 2010); larval development and/or growth in crustaceans (Arnold et al. 2009; Findlay et al. 2009, 2010;
McDonald et al. 2009), molluscs (Kurihara et al. 2007; Ellis et al. 2009; Parker et al. 2009), corals (Albright et al. 2008, 2010; Cohen et al. 2009; Suwa et al. 2010; Albright & Langdon 2011) and echinoderms (Kurihara & Shirayama 2004; Dupont et al. 2008; Clark et al. 2009; Sheppard Brennand et al. 2010; O’Donnell et al. 2010); physiology and behavior of molluscs (Ellis et al. 2009); survival of echinoderms (Dupont et al. 2008; Clark et al. 2009) and crustaceans (Findlay et al. 2009); stress response in sea urchins (O’Donell et al. 2009; Todgham & Hofmann 2009); and gene expression in sea urchins (Todgham & Hofmann 2009; O’Donnell et al. 2010). A compilation of recent studies investigating the effects of ocean acidification on early life history stages of marine invertebrates is presented in Table 1.1. This listing is restricted to comprehensive studies for which methods, data, and statistical analyses are available and is primarily focused on studies conducted over the last decade, under the context of near-future ocean acidification scenarios. Despite this recent surge in activity, the majority of these studies have been conducted on echinoderms, molluscs, and crustaceans, and comparatively few studies focus on the potential response(s) of early life history stages of corals. As coral recruitment, post-settlement survivorship and growth are critical to reef persistence and resilience, it is important to better understand the repercussions of climate change scenarios on such factors.
Table 1.1. Compilation of recent studies investigating the effects of ocean acidification (pH change) on early life history stages of marine invertebrates. This listing is restricted to comprehensive studies for which methods, data, and statistical analyses are available and is focused on studies conducted over the last decade, under the context of near-future ocean acidification scenarios.

<table>
<thead>
<tr>
<th>Taxon</th>
<th>CO\textsubscript{2} or HCl (ppm)</th>
<th>pH</th>
<th>Temp \degree{Celsius}</th>
<th>Exposure period</th>
<th>Effect</th>
<th>Source</th>
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<tbody>
<tr>
<td><strong>Crustacea</strong></td>
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<tr>
<td>Acartia steueri</td>
<td>CO\textsubscript{2} (365, 2365, 10,365)</td>
<td>6.8, 7.3, 8.1</td>
<td>27</td>
<td>8 d</td>
<td>Decreased egg production rate at 10,365 ppm (reversible effect)</td>
<td>Kurihara et al. 2004</td>
</tr>
<tr>
<td>A. erythraea</td>
<td>CO\textsubscript{2} (365, 5365, 10,365)</td>
<td>6.8, 7.0, 8.1</td>
<td>27</td>
<td>8 d</td>
<td>Decreased egg production rate; decreased hatching rate and increased mortality of nauplii (embryos)</td>
<td>Kurihara et al. 2004</td>
</tr>
<tr>
<td>Homarus gammarus</td>
<td>CO\textsubscript{2} (315, 1200)</td>
<td>8.1, 8.4</td>
<td>17</td>
<td>28 d</td>
<td>Reduced mass during final developmental stage; reduced Ca\textsuperscript{2+} and Mg\textsuperscript{2+} content; no effect on length, development, or survivorship</td>
<td>Arnold et al. 2009</td>
</tr>
<tr>
<td>Semibalanus balanoides</td>
<td>CO\textsubscript{2} (346, 922)</td>
<td>7.70, 8.07</td>
<td>12</td>
<td>104 d</td>
<td>Slowed embryonic development; no effect on [Ca\textsuperscript{2+}] in shells; decreased adult survivorship, altered shell mineralogy</td>
<td>Findlay et al. 2009</td>
</tr>
<tr>
<td>Amphibalanus amphitrite</td>
<td>CO\textsubscript{2}</td>
<td>7.4, 8.2</td>
<td>25-28</td>
<td>11 wk</td>
<td>Increased calcification with simultaneous dissolution; decreased breaking strength; no effect on larval condition, size, or survival</td>
<td>McDonald et al. 2009</td>
</tr>
<tr>
<td>S. balanoides</td>
<td>CO\textsubscript{2} (380, 1000)</td>
<td>7.70, 8.07</td>
<td>14, 19</td>
<td>30 d</td>
<td>No effect on growth; decreased survivorship and [Ca\textsuperscript{2+}] in shells</td>
<td>Findlay et al. 2010</td>
</tr>
<tr>
<td>Elminius modestus</td>
<td>CO\textsubscript{2} (380, 1000)</td>
<td>7.70, 8.07</td>
<td>14, 19</td>
<td>30 d</td>
<td>Decreased growth; no effect on survivorship or [Ca\textsuperscript{2+}] in shells</td>
<td>Findlay et al. 2010</td>
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<td><strong>Cnidaria</strong></td>
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<tr>
<td>Porites astreoides</td>
<td>HCl (ambt., 560, 700)</td>
<td>7.8, 7.9, 8.0</td>
<td>25.4, 26.6</td>
<td>21, 28 d</td>
<td>Reduced growth (lateral extension); no direct effect on settlement</td>
<td>Albright et al. 2008</td>
</tr>
<tr>
<td>A. digiflata</td>
<td>CO\textsubscript{2}</td>
<td>6.55-8.03</td>
<td>26.8 minutes</td>
<td></td>
<td>Decreased sperm motility at pH &lt; 7.8</td>
<td>Morita et al. 2009</td>
</tr>
<tr>
<td>Favia fragum</td>
<td>HCl</td>
<td>7.54, 7.87, 8.04, 8.17</td>
<td>25</td>
<td>8 d</td>
<td>Delayed onset of calcification; decreased primary polyp growth; altered crystal morphology and composition</td>
<td>Cohen et al. 2009</td>
</tr>
<tr>
<td>Acropora spp.</td>
<td>CO\textsubscript{2} (400-475, 905-1660, 2115-3585)</td>
<td>7.3, 7.6, 8.0</td>
<td>26.8</td>
<td>4 d</td>
<td>Reduced polyp growth &amp; algal infection rates; no effect on survival</td>
<td>Suwa et al. 2010</td>
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<tr>
<td>A. palmata</td>
<td>CO\textsubscript{2} (436-491, 599-673, 876-998)</td>
<td>7.8, 7.9, 8.0</td>
<td>28.0-28.2</td>
<td>50 d</td>
<td>Reduced fertilization (dependent on sperm concentration); reduced post-metamorphic settlement (indirect effect); reduced growth</td>
<td>Albright et al. 2010</td>
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<tr>
<td>P. astreoides</td>
<td>CO\textsubscript{2} (380, 560, 800)</td>
<td>7.8, 7.9, 8.0</td>
<td>26-28</td>
<td>49 d</td>
<td>Reduced respiration of planulae; reduced settlement (indirect effect); reduced growth (lateral extension)</td>
<td>Albright &amp; Langdon 2011</td>
</tr>
<tr>
<td>P. panamensis</td>
<td>CO\textsubscript{2} (487-546, 861-1006)</td>
<td>7.83, 8.05</td>
<td>28.4, 29.5</td>
<td>42 d</td>
<td>No effect on settlement or survivorship; low pH decreased growth, exacerbated with 1\degree{Celsius} warming</td>
<td>Anlauf et al. 2011</td>
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<tr>
<td>A. digitifera</td>
<td>CO\textsubscript{2} (331-397, 1172-1685, 2011-3100)</td>
<td>7.3, 7.6, 8.0</td>
<td>26.3</td>
<td>3, 7 d</td>
<td>Decreased O\textsubscript{2} consumption by planulae; reduced metamorphosis</td>
<td>Nakamura et al. 2011</td>
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<td>Taxon</td>
<td>CO₂ or HCl (ppm)</td>
<td>pH</td>
<td>Temp °C</td>
<td>Exposure period</td>
<td>Effect</td>
<td>Source</td>
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<tr>
<td>Mollusca</td>
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<tr>
<td>Crassostrea gigas</td>
<td>CO₂ (348, 2268)</td>
<td>7.4, 8.2</td>
<td>23</td>
<td>48 h</td>
<td>Abnormal, reduced, and delayed development; decreased shell mineralization; reduced larval size</td>
<td>Kurihara et al. 2007</td>
</tr>
<tr>
<td>C. gigas</td>
<td>CO₂</td>
<td>7.8, 8.15</td>
<td>21-22 minutes</td>
<td>No effect on sperm motility, velocity or fertilization kinetics</td>
<td>Havenhand &amp; Schlegel 2009</td>
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<tr>
<td>C. gigas</td>
<td>CO₂ (375, 600, 750, 1000)</td>
<td>7.7-8.0</td>
<td>18, 22, 26, 30</td>
<td>4 d</td>
<td>Reduced fertilization, development, larval and spat size at elevated pCO₂ and suboptimal temperatures; malformation</td>
<td>Parker et al. 2010</td>
</tr>
<tr>
<td>Mytilus galloprovincialis</td>
<td>CO₂ (ambient, 2000)</td>
<td>7.42, 8.13</td>
<td>13</td>
<td>6 d</td>
<td>No effect on embryogenesis; delayed onset of calcification; shell malformation; reduced size</td>
<td>Kurihara et al. 2008</td>
</tr>
<tr>
<td>Saccostrea glomerata</td>
<td>HCl (375, 600, 750, 1000)</td>
<td>7.7-8.0</td>
<td>18, 22, 26, 30</td>
<td>48 h</td>
<td>Decreased fertilization; decreased larval number and size; increased abnormalities</td>
<td>Parker et al. 2009</td>
</tr>
<tr>
<td>S. glomerata</td>
<td>CO₂ (220, 509, 776)</td>
<td>7.6, 7.8, 8.1</td>
<td>26</td>
<td>8 d</td>
<td>Decreased larval survivorship, development, and growth; evidence of shell malformation/dissolution</td>
<td>Watson et al. 2009</td>
</tr>
<tr>
<td>S. glomerata</td>
<td>CO₂ (375, 600, 750, 1000)</td>
<td>7.7-8.0</td>
<td>18, 22, 26, 30</td>
<td>4 d</td>
<td>Reduced fertilization, development, larval and spat size at elevated pCO₂ and suboptimal temperatures; malformation</td>
<td>Parker et al. 2010</td>
</tr>
<tr>
<td>Crassostrea virginica</td>
<td>CO₂ (380, 3500)</td>
<td>7.5, 8.2</td>
<td>20</td>
<td>20 wk</td>
<td>Increased metabolism and mortality, decreased shell and soft-body growth; increased shell thickness; reduced hardness</td>
<td>Beniash et al. 2010</td>
</tr>
<tr>
<td>C. virginica</td>
<td>CO₂ (280, 380, 560, 800)</td>
<td>7.76, 7.91, 8.06, 8.2</td>
<td>25</td>
<td>28 d</td>
<td>Decreased shell area and calcium content. Net calcification (albeit slowed) when Ω_{arag} &lt; 1</td>
<td>Miller et al. 2009</td>
</tr>
<tr>
<td>C. virginica</td>
<td>CO₂ (350, 665, 1535)</td>
<td>7.5, 7.85, 8.0-8.1</td>
<td>24</td>
<td>20 d</td>
<td>Decreased size; delayed metamorphosis; reduced survivorship at 1535 ppm</td>
<td>Talmage &amp; Gobler 2009</td>
</tr>
<tr>
<td>Crassostrea ariakensis</td>
<td>CO₂ (280, 380, 560, 800)</td>
<td>7.79, 7.92, 8.08, 8.17</td>
<td>25</td>
<td>28 d</td>
<td>No effect on growth or calcification. Net calcification at Ω_{arag} &lt; 1</td>
<td>Miller et al. 2009</td>
</tr>
<tr>
<td>Mercenaria mercenaria</td>
<td>CO₂ (350, 665, 1535)</td>
<td>7.5, 7.8, 8.0</td>
<td>24</td>
<td>20 d</td>
<td>Decreased size and survivorship, delayed metamorphosis</td>
<td>Talmage &amp; Gobler 2009</td>
</tr>
<tr>
<td>M. mercenaria</td>
<td>CO₂ (250, 390, 750, 1500)</td>
<td>7.5, 7.8, 8.05, 8.2</td>
<td>24</td>
<td>36 d</td>
<td>Decreased growth, metamorphosis, survival, lipid accumulation; reduced shell thickness; shell malformation</td>
<td>Talmage &amp; Gobler 2010</td>
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<tr>
<td>Mercenaria spp.</td>
<td>CO₂ (424, 1120, 1950)</td>
<td>7.4, 7.6, 8.0</td>
<td>20</td>
<td>8 h</td>
<td>Decreased calcification - size dependent pH effect</td>
<td>Waldbusser et al. 2010</td>
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<tr>
<td>Argopecten irradians</td>
<td>CO₂ (350, 665, 1535)</td>
<td>7.5, 7.85, 8.0-8.1</td>
<td>24</td>
<td>20 d</td>
<td>Decreased size and survivorship, delayed metamorphosis</td>
<td>Talmage &amp; Gobler 2009</td>
</tr>
<tr>
<td>A. irradians</td>
<td>CO₂ (250, 390, 750, 1500)</td>
<td>7.5, 7.8, 8.05, 8.2</td>
<td>24</td>
<td>38 d</td>
<td>Decreased growth, metamorphosis, survival, lipid accumulation; reduced shell thickness; shell malformation</td>
<td>Talmage &amp; Gobler 2010</td>
</tr>
<tr>
<td>Taxon</td>
<td>CO₂ or HCl (ppm)</td>
<td>pH</td>
<td>Temp °C</td>
<td>Exposure period</td>
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<tr>
<td>Mytilus edulis</td>
<td>CO₂, (460-640, 1100-1200)</td>
<td>7.8, 8.1</td>
<td>16, 19</td>
<td>15 d</td>
<td>Altered development; slowed growth (length and thickness); no effect on hatching or mortality</td>
<td>Gazeau et al. 2010</td>
</tr>
<tr>
<td>Strombus luhuanus</td>
<td>CO₂ (ambt., 560)</td>
<td>7.90, 7.94</td>
<td>&lt;30</td>
<td>6 mo</td>
<td>Decreased growth (wet weight) at wk 8; decreased shell height at wk 12; increased mortality in 1 of 2 experiments</td>
<td>Shirayama &amp; Thornton 2005</td>
</tr>
<tr>
<td>Littorina obtusata</td>
<td>CO₂ (ambt.,1100)</td>
<td>7.6, 8.1</td>
<td>15</td>
<td>24 d</td>
<td>Reduced egg viability; slowed development; reduced heart rate, motility, rotation rates; altered shell morphology</td>
<td>Ellis et al. 2009</td>
</tr>
<tr>
<td>Echinometra mathaei</td>
<td>HCl &amp; CO₂ (860-10,360)</td>
<td>6.8-7.8</td>
<td>3 d</td>
<td>pH &lt; 7.8, skeletal malformation, reduced larval size &amp; fertilization</td>
<td>Kurihara &amp; Shirayama 2004</td>
<td></td>
</tr>
<tr>
<td>E. mathaei</td>
<td>CO₂ (ambt., 560)</td>
<td>7.90, 7.94</td>
<td>&lt;30</td>
<td>6 mo</td>
<td>Decreased growth at 12 wk; mortality in 1 of 2 expts.</td>
<td>Shirayama &amp; Thornton 2005</td>
</tr>
<tr>
<td>Hemicentrotus pulcherrimus</td>
<td>HCl &amp; CO₂ (860-10,360)</td>
<td>6.8-7.8</td>
<td>3 d</td>
<td>pH &lt; 7.8, skeletal malformation, reduced larval size &amp; fertilization</td>
<td>Kurihara &amp; Shirayama 2004</td>
<td></td>
</tr>
<tr>
<td>H. pulcherrimus</td>
<td>CO₂ (ambt., 560)</td>
<td>7.90, 7.94</td>
<td>&lt;30</td>
<td>6 mo</td>
<td>Decreased growth at 14 wk; mortality in 1 of 2 expts.</td>
<td>Shirayama &amp; Thornton 2005</td>
</tr>
<tr>
<td>Heliocidaris erythrogramma</td>
<td>CO₂ (ambt.,1000)</td>
<td>7.7, 8.1</td>
<td>20.5</td>
<td>24 h</td>
<td>Decreased sperm motility, swimming speed, and fertilization</td>
<td>Havenhand et al. 2008</td>
</tr>
<tr>
<td>H. erythrogramma</td>
<td>CO₂ (250-690)</td>
<td>7.6-8.2</td>
<td>20-26</td>
<td>4 d</td>
<td>No effect of CO₂ on fertilization or development; T effect on development, not fertilization; no T*CO₂ interaction</td>
<td>Byrne et al. 2009</td>
</tr>
<tr>
<td>H. erythrogramma</td>
<td>CO₂ (370, 1100, 1850)</td>
<td>7.6-8.2</td>
<td>20-26</td>
<td>2 h</td>
<td>No effect of T or CO₂, only sperm concentration</td>
<td>Byrne et al. 2010</td>
</tr>
<tr>
<td>Strongylocentrotus franciscanus</td>
<td>CO₂ (400, 800, 1800)</td>
<td>7.6, 7.8, 8.0</td>
<td>10</td>
<td>3 h</td>
<td>Decreased fertilization efficiency; increased susceptibility to polyspermy at 1580 ppm; CO₂ effect dependent on sperm concentration</td>
<td>Reuter et al. 2010</td>
</tr>
<tr>
<td>S. franciscanus</td>
<td>CO₂ (380, 540, 970)</td>
<td>7.87, 7.98, 8.04</td>
<td>15, 19, 21, 23, 25, 27, 29, 31</td>
<td>~96 h</td>
<td>Gametes (larvae) fertilized (reared) at elevated CO₂ showed reduced expression of heat shock proteins in response to acute T stress (1h); T of max. induction was shifted</td>
<td>O'Donnell et al. 2009</td>
</tr>
<tr>
<td>Strongylocentrotus purpuratus</td>
<td>CO₂ (380, 540, 1020)</td>
<td>7.88, 7.96, 8.01</td>
<td>15</td>
<td>&lt; 72 h</td>
<td>Decreased gene expression in 4 major cellular processes: biomineralization, cellular stress response, metabolism, apoptosis</td>
<td>Todgham &amp; Hofmann 2009</td>
</tr>
<tr>
<td>Tripneustes gratilla</td>
<td>CO₂ (450, 1200, 1900)</td>
<td>8.15, 7.8, 7.6</td>
<td>24, 27, 30</td>
<td>5 d</td>
<td>pCO₂ decreased larval growth; T &lt; 30°C increased growth; interaction of CO₂ *T - 3° warming (24°-27°) diminished CO₂ effect</td>
<td>Sheppard Brennand et al. 2010</td>
</tr>
<tr>
<td>Taxon</td>
<td>CO₂ or HCl (ppm)</td>
<td>pH</td>
<td>Temp °C</td>
<td>Exposure period</td>
<td>Effect</td>
<td>Source</td>
</tr>
<tr>
<td>-------</td>
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</tr>
<tr>
<td>Tripneutes gratilla (tropical spp.)</td>
<td>CO₂ (395-1119)</td>
<td>6.0, 6.5, 7.0, 7.5, 7.7, 7.8</td>
<td>26</td>
<td>4 d</td>
<td>Decreased calcification and size; no effect on fine skeletal morphology; decreased survival at pH &lt; 7.0</td>
<td>Clark et al. 2009</td>
</tr>
<tr>
<td>Pseudechinus huttoni (temp. spp.)</td>
<td>CO₂ (429-1,282)</td>
<td>6.0, 6.5, 7.0, 7.5, 7.7, 7.8</td>
<td>10-15</td>
<td>9 d</td>
<td>Decreased calcification and size; degradation of fine skeletal morphology; decreased survival at pH &lt; 7.0</td>
<td>Clark et al. 2009</td>
</tr>
<tr>
<td>Euechinus chloroticus (temp. spp.)</td>
<td>CO₂ (438-1,320)</td>
<td>6.0, 6.5, 7.0, 7.5, 7.7, 7.8</td>
<td>10-15</td>
<td>13 d</td>
<td>Decreased calcification and size; degradation of fine skeletal morphology; decreased survival at pH &lt; 7.0</td>
<td>Clark et al. 2009</td>
</tr>
<tr>
<td>Sterechinus neumayeri (polar spp.)</td>
<td>CO₂ (521-1,380)</td>
<td>6.0, 6.5, 7.0, 7.5, 7.7, 7.8</td>
<td>-1</td>
<td>7 d</td>
<td>No effect on calcification; no effect on fine skeletal morphology; decreased survival at pH &lt; 7.0</td>
<td>Clark et al. 2009</td>
</tr>
<tr>
<td>Lytechinus pictus</td>
<td>CO₂ (380, 540, 970)</td>
<td>7.78, 7.87, 7.93</td>
<td>18.5</td>
<td>6 d</td>
<td>Altered larval size, shape; down-regulation of genes central to energy metabolism, biomineralization; up-regulation of genes involved in ion regulation and acid-base balance pathways</td>
<td>O'Donnell et al. 2010</td>
</tr>
<tr>
<td>Ophiothrix fragilis - (seastar)</td>
<td>CO₂</td>
<td>7.7, 7.9, 8.1</td>
<td>14</td>
<td>8 d</td>
<td>100% mortality within 8 days (mortality starting at 4-6 days). Decrease in larval size; abnormal and slowed development and skeletogenesis (asymmetry, altered skeletal proportions)</td>
<td>Dupont et al. 2008</td>
</tr>
<tr>
<td>Crossaster papposus (seastar)</td>
<td>CO₂ (372-930)</td>
<td>7.7, 8.1</td>
<td>12</td>
<td>38 d</td>
<td>Increased growth and developmental rate at low pH; no effect on survival or skeletogenesis</td>
<td>Dupont et al. 2010</td>
</tr>
<tr>
<td>Pisaster ochraceus</td>
<td>CO₂ (380, 780)</td>
<td>7.8, 7.9</td>
<td>12, 15</td>
<td>70 d</td>
<td>Increased growth and feeding at increased pCO₂; decreased relative calcified mass at 12°C; 3°C warming offset CO₂ effect</td>
<td>Gooding et al. 2009</td>
</tr>
<tr>
<td>Holothuria spp. (Sea cucumber)</td>
<td>CO₂ (400-21,000)</td>
<td>6.6, 7.3, 7.6, 7.7, 7.8, 8.0</td>
<td>26.8 minutes</td>
<td></td>
<td>Reduced sperm motility at pH ≤ 7.7</td>
<td>Morita et al. 2009</td>
</tr>
</tbody>
</table>
Intergovernmental Panel on Climate Change Scenarios

The Fourth Assessment Report (AR4) released by the Intergovernmental Panel on Climate Change (IPCC) includes six modeling groups, formulated by the Special Report Emissions Scenarios (SRES), which have produced 40 separate emissions scenarios. Each modeling group is based on different drivers, the primary considerations being demographics (e.g., land-use changes), population projection and economic/technological development (e.g., alternative sources of energy). Results of all scenarios span a wide range; for example, projected CO₂ emissions in 2100 range from less than 6 GtC, approximately the same emissions level as in 1990, to more than 40 GtC, a sevenfold increase. According to the six main scenarios (A1F1, A1T, A2, B2, B1, and A1B), pCO₂ levels will likely attain 560 ppm (2x pre-industrial values) by the middle of this century, and 800-1000 ppm is well within the range of values projected for the end of the century, year 2100 (Figure 1.3) (IPCC 2007). These mid-century and end-of-century pCO₂ values are widely used in laboratory-based experiments evaluating the effect of ocean acidification on organism biology and physiology. In the experiments detailed in the following chapters, three pCO₂ values were targeted to approximate present day (ambient, 380 ppm), mid-century (560 ppm) and end of century (800-1000 ppm) pCO₂ levels.

Experimental Systems for the Manipulation of Carbonate Chemistry

The following chapters present the findings of a series of experiments conducted from 2007-2010 investigating the effects of increasing pCO₂ on three sequential life history stages that are critical to coral recruitment: (1) pre-settlement ecology (fertilization and larval availability); (2) settlement ecology; and (3) post-settlement
ecology (juvenile growth). Chapter 2 investigates the effects of ocean acidification (OA) on the sperm swimming speed and fertilization success of two common Caribbean broadcast-spawning corals, *Acropora palmata* and *Montastraea faveolata*. Chapter 3 investigates the effects of OA on larval settlement by investigating larval physiology and settlement behavior. Larval physiology is evaluated by measuring respiration rates and C/N ratios of the common Caribbean coral *Porites astreoides*. Settlement behavior is evaluated by testing direct (physiological disruption of metamorphosis) and indirect (interference with settlement via benthic habitat cues) effects of $pCO_2$ on the successful settlement and metamorphosis of planulae of two common Caribbean coral species, *P. astreoides* (brooder) and *A. palmata* (spawner). Chapter 4 investigates the effects of OA on post-settlement growth in four coral species, a spawning species, *A. palmata*, and three brooding species, *P. astreoides*, *Favia fragum*, and *Agaricia agaricites*.

The experiments detailed in the following chapters were conducted in one of two experimental systems. In 2007, experiments were conducted in an indoor experimental facility in which seawater carbonate chemistry was manipulated via hydrochloric acid additions. In 2008-2010, experiments were conducted in an outdoor greenhouse in which seawater carbonate chemistry was manipulated via direct bubbling with carbon dioxide (CO$_2$) gas. There is a fundamental chemical difference between these two approaches that could, in theory, influence the biological response: the addition of HCl to seawater reduces the total alkalinity, TA, while leaving total dissolved inorganic carbon, DIC, constant; adding CO$_2$ to seawater increases the DIC while TA remains constant. The addition of Cl ions in the first treatment is assumed to be insignificant as this ion already dominates those in seawater. In both scenarios, pH decreases while CO$_2_{aq}$ and $pCO_2$
increase. The utility of the acid-addition methodology has been a topic of recent controversy in the scientific community, as it is thought that some species respond differently to acidified conditions when DIC is elevated. Indeed, Kurihara et al. (2004) showed that the fertilization rate of two species of sea urchins was more sensitive to CO$_2$-induced acidification than HCl-induced acidification. The permeability of CO$_2$ across cell membranes may be responsible for this heightened sensitivity (Kurihara et al. 2004). Ultimately, manipulating carbonate chemistry via bubbling with CO$_2$ is the preferred method of seawater chemistry manipulation for ocean acidification experiments as it better approximates the global uptake of fossil fuel CO$_2$ by the oceans. For this reason, experiments in later years (2008-2010) were restricted to the CO$_2$-manipulated seawater system. Details of each experimental system are described below.

**Hydrochloric Acid System**

In 2007, an indoor flow-through seawater system was used to create and maintain three aragonite saturation states: $\Omega_{\text{arag}} = 3.2$ (control), $\Omega_{\text{arag}} = 2.6$ (mid), and $\Omega_{\text{arag}} = 2.2$ (low) (based on projected $p$CO$_2$ scenarios for the years 2065 and 2100, respectively, as determined by the Intergovernmental Panel on Climate Change (IPCC) 3rd Assessment Report (IPCC 2001). Seawater from Bear Cut, Virginia Key, FL was pumped into a 240,000 l settling tank, filtered through sand to remove particulate matter, and piped to three tanks where the carbonate system was manipulated. Total alkalinity (TA) and pH were adjusted via constant-drip 1M HCl additions and control of seawater flow rates. Treated water was then introduced to experimental aquaria (~18 l) at a constant rate. Duplicate aquaria were used for each treatment. Water temperature was maintained at a
constant temperature by use of heating elements and/or chillers. Schematic and photographic representations of this system are presented in Figure 1.6.

**Carbon Dioxide Gas System**

In 2008-2010, an outdoor seawater facility at the University of Miami’s Experimental Hatchery (UMEH) was used to target three $p$CO$_2$ levels: 380 ppm (control); 560 ppm; and 800-1,000 ppm (based on projected $p$CO$_2$ scenarios for the years 2065 and 2100, respectively, as determined by the Intergovernmental Panel on Climate Change 4th Assessment Report (IPCC 2007). The experimental facility consisted of nine 60 l aquaria arranged in ranks of three, in three 2 m long fiberglass tanks. Each aquarium was connected to its own 200 l sump tank. The CO$_2$ level of the feed seawater to the aquaria was controlled by bubbling the water in the 200 l sump tanks with ambient air or a mixture of ambient air and pure CO$_2$ gas. The mixture of air and CO$_2$ gas was achieved using mass flow controllers. Natural seawater from Bear Cut, Virginia Key, FL was pumped through a sand filter to remove particulate matter, into a holding tank, and piped to the experimental system. Fresh seawater flowing at a continuous rate turned over the combined volume (260 l) approximately once per day. Temperature was controlled by chilled water (26°C) that flowed through coils in each fiberglass tank and heating elements. Tanks were exposed to natural, outdoor lighting with UV filtration. Schematic and photographic representations of this system are presented in Figure 1.7.
Figure 1.6. Schematic design (a) and photograph (b) of experimental seawater system for investigation of reef larvae under controlled saturation state (HCl manipulations). Six ~18 l treatment aquaria running three saturation state treatments ($\Omega_{arag} = 3.5$ (ambient), $\Omega_{arag} = 3.1$, and $\Omega_{arag} = 2.6$). Refer to text for details.
Figure 1.7. Schematic diagram (courtesy of C. Langdon and A. Baker) (a) and photographs (b,c) of experimental facility at UMEH for culturing corals under controlled temperature and CO$_2$ conditions (CO$_2$-manipulated carbonate chemistry). The blue boxes outline aquaria operated at the same $p$CO$_2$ level. All aquaria were held at the same temperature for experiments described here. Refer to text for details.
Carbonate Chemistry Analyses

For all experiments detailed in the following chapters, seawater carbonate chemistry was monitored by use of discrete water samples. Water samples were analyzed for total alkalinity (TA) and pH. TA was determined in duplicate (30-40 ml analyses) using an automated, open-cell Gran titration (Dickson et al. 2007, SOP3b), and accuracy was checked against certified seawater reference standards (A. Dickson, Scripps Institute of Oceanography). pH was determined on the total scale using an Orion Ross combination pH electrode calibrated at 25ºC against a seawater TRIS buffer (Dickson et al. 2007, SOP6). Total DIC, $p$CO$_2$, concentrations of HCO$_3^-$, CO$_3^{2-}$, CO$_2$, and $\Omega_{arag}$ were computed from TA, pH, temperature, and salinity using the program CO2SYS (E. Lewis, Brookhaven National Laboratory), with dissociation constants for carbonate determined by Mehrbach et al. (1973), as refit by Dickson & Millero (1987) and dissociation constant for boric acid determined by Dickson (Dickson 1990). pH is reported on the total scale, the scale on which K1 and K2 were determined.
Chapter 2: Effects of Ocean Acidification on Sexual Reproduction

Summary

The effect of ocean acidification (OA) on sexual reproduction of reef-building corals is largely unknown. To determine whether OA threatens coral sexual reproduction, fertilization and sperm swimming speeds of two species of Caribbean broadcast-spawning corals, *Acropora palmata* and *Montastraea faveolata*, were tested at $pCO_2$ levels that represent average ambient conditions during coral spawning (~400 µatm) and the range of $pCO_2$ increases that are expected to occur in this century [~560 µatm (mid-CO$_2$) and ~800 µatm (high-CO$_2$)]. *Acropora palmata* gametes were collected from Elbow Reef, and *M. faveolata* gametes were collected from Grecian Rocks. To characterize chemical conditions experienced by gametes in situ, water chemistry was monitored at the two spawning reefs over the course of 24 hours. Results of sperm swimming speed analyses were inconclusive. However, for both species, fertilization was negatively impacted by increasing $pCO_2$, and impairment of fertilization was exacerbated at lower sperm concentrations. These results suggest that OA has the potential to impact sexual reproduction of these species by reducing the larval supply and compromising downstream life history stages such as settlement and post-settlement growth and survivorship.

Background

Sexual reproduction in scleractinian reef corals is important for maintaining genetic diversity, populating denuded areas, determining the community structure of coral reefs, and replenishing reefs post disturbances. Larval supply to a reef depends on
gamete production (fecundity), fertilization success, and larval transport (i.e., dispersal and connectivity) (Ritson-Williams et al. 2009). Reef-building corals have two primary strategies for sexual reproduction: (1) brooding, in which sperm are released into the water column and taken in by conspecifics for internal fertilization; and (2) broadcast spawning, in which eggs and sperm are released into the environment for external fertilization (Harrison 2011). The majority of reef-building corals are hermaphroditic broadcast spawners: they release gamete bundles, containing both eggs and sperm, into the water column and are reliant on external fertilization and larval development. Coral larvae spend hours to days developing in the water column before they are capable of settling on the reef.

As broadcast spawners are typically reliant on one to two planulating cycles per year, it is critical that fertilization be successful (Ritson-Williams et al. 2009). Fertilization success depends on a variety of factors, including sperm density, gamete age, water temperature, oxygen-availability, salinity, and nutrients, pH, and $p$CO$_2$ (reviewed in Ritson-Williams et al. 2009; Reuter et al. 2010). External fertilization and development renders gametes and larvae particularly vulnerable to the chemistry of the surrounding water column. As the acidity of the water has already increased ~30% over the last 100 years, it is critical to understand the repercussions of changing ocean chemistry on sexual reproduction.

Recent work indicates negative effects of ocean acidification (OA) on fertilization success of some marine invertebrates, including oysters (Parker et al. 2009, 2010; but see Havenhand & Schlegel 2009), and urchins (Kurihara & Shirayama 2004; Havenhand et al. 2008; Reuter et al. 2010; but see Byrne et al. 2010). However, little is known
regarding the effect(s) of acidification on sexual reproduction in corals. To determine whether OA threatens the successful sexual reproduction of reef-building corals, fertilization and sperm swimming speeds of two species of Caribbean broadcast-spawning corals, *Acropora palmata* and *Montastraea faveolata*, were tested at $pCO_2$ levels that represent average ambient conditions during coral spawning (~400 µatm) and the range of $pCO_2$ increases that are expected to occur in this century [~560 µatm (mid-$CO_2$) and ~800 µatm (high-$CO_2$)]. To characterize chemical conditions experienced by gametes in situ, water chemistry was monitored at the two spawning reefs over the course of 24 hours near predicted spawning nights.

**Materials and Methods**

*Study Site and Species*

The experimental corals chosen for fertilization studies were two ecologically significant Caribbean framework builders: the elkhorn coral *Acropora palmata* and the mountainous star coral *Montastraea faveolata*. *Acropora palmata* is a fast-growing, branching species that has historically functioned as a primary framework builder on many Caribbean shallow reefs. However, Caribbean acroporid populations have experienced widespread decline over the last several decades due to hurricanes, disease, bleaching, and predation (Bruckner 2002; Gardner et al. 2003). A drastic reduction in population size resulted in the 2006 designation of *A. palmata* as a threatened species under the US Endangered Species Act (NMFS 2006). Recovery of *A. palmata* populations will likely require high rates of recruitment (Bruckner 2002; Szmant & Miller 2006; Williams et al. 2008). Although this coral’s primary reproductive mode is
asexual fragmentation, sexual recruitment is critical for maintaining the genetic diversity of future populations. *Montastraea faveolata* is a slow-growing framework builder that is commonly found in shallow reef environments throughout the Caribbean. While this species has not seen population declines as severe as Caribbean acroporids, it is nevertheless threatened and is currently being considered, along with 81 other coral species, to be listed on the US Endangered Species Act alongside *A. palmata* and *A. cervicornis*.

Coral spawning activity of *A. palmata* and *M. faveolata* was monitored from 2008-2010. Monitoring took place on Elbow Reef for *A. palmata* and Grecian Rocks for *M. faveolata* (Figure 2.1). The Elbow is a bank reef with a well-defined spur-and-groove system. It is a Sanctuary Preservation Area (SPA) that is located approximately 10km (5.5 nm) southeast of Key Largo. Grecian Rocks, another SPA, is located slightly south of Elbow Reef off the coast of Key Largo. *Acropora palmata* colonies were monitored on nights 2-5 following the full moon in August, and *M. faveolata* colonies were monitored on nights 5-8 following the full moon in August/September. *Acropora palmata* typically spawns at ~22:00-22:15, while *M. faveolata* typically spawns at ~ 22:45-23:00.

**Carbonate Chemistry in Reef Waters**

Through the support of projects and/or experiments such as the Bermuda Atlantic Time-series Study (BATS; Bates et al. 1996; Bates 2007) Climate Variability and Predictability (CLIVAR; part of the World Climate Research Program or WCRP), CO₂ repeat hydrography cruises, the World Ocean Circulation Experiment (WOCE) and Joint Global Ocean Flux Study (JGOFS; Feely et al. 2004; Sabine et al. 2004), the chemistry of
Figure 2.1. Map of the Florida Keys showing the location of the two study sites: Elbow Reef for collection of *Acropora palmata* spawn and Grecian Rocks for collection of *Montastraea faveolata* spawn (www.floridakeys.noaa.gov).

The global ocean’s surface waters has been documented over the course of several decades, and fluctuations in these data are being used to characterize changes in chemistry due to increasing levels of atmospheric CO$_2$. The majority of these surveys and cruises have targeted longitudinal transects across open ocean waters in both the Pacific and Atlantic Oceans, and extrapolation of these datasets to coastal waters can result in significant errors (D. Feely, *pers. comm.*). There is a paucity of information documenting and characterizing trends in the carbonate chemistry of near-shore, shallow reef waters.
To place the following experiments in better context with respect to in situ carbonate chemistry, reef water was sampled at the two spawning reefs, Elbow Reef and Grecian Rocks, over a 24-hour period to characterize the mean daily values of various carbonate parameters and the range in values experienced over the course of the day. Discrete water samples were collected from surface waters approximately 1-2 weeks after spawning in August 2009. At Elbow Reef, *A. palmata* colonies spawned August 10-12, 2009. Sampling commenced 9 days post-spawning at 21:30 on August 21, and samples were collected approximately every hour until 19:30 on August 22. A total of 17 samples were collected. At Grecian Rocks, *M. faveolata* colonies spawned August 13-14, 2009. Sampling commenced 8 days post-spawning at 16:00 on August 22, and samples were collected approximately every hour until 15:00 on August 23. A total of 17 samples were collected. Temperature and salinity of the water were noted at the time of sampling. Water samples were analyzed according to the methodology outlined in Chapter 1, ‘Carbonate Chemistry Analyses’. Output temperatures (i.e., in situ temperature at the time of sampling) were obtained from temperature loggers maintained by the Benthic Ecosystems Assessment Research (BEAR) Unit at the National Marine Fisheries Service (NMFS).

Data were used to characterize mean values and diurnal ranges for various carbonate chemistry parameters on the study reefs and relate these values to those that were used for experimental purposes. Diurnal fluctuations in pH, \( p\text{CO}_2 \), DIC, HCO\(_3^-\), CO\(_3^{2-}\), CO\(_2\), and \( \Omega_{\text{arag}} \) follow a cyclical pattern that approximates a periodic wave; for this reason, data for these parameters were fit to sine models of the form \( y = Asin(Bx + C) + D \) where: ‘A’ is the amplitude of the function, ‘B’ is the frequency (\( 2\pi/B \) is the period),
‘C’/B’ is the phase shift, and ‘D’ is the vertical displacement of the mid point of the function above the x-axis. Natural diurnal fluctuations in carbonate parameters are likely to deviate from a perfect periodic sine wave due to variation in day/night cycles. A sine function assumes equal periods of illumination and darkness (12 h), and depending on the time of year, shorter/longer periods of daylight may alter the curve; however, as the study sites are located near the equator where seasonal fluctuations in day length are small, the sine function may closely approximate the diurnal fluctuations of these parameters.

As water samples were taken at the surface, it is possible that the chemical conditions near the benthos differed due to stratification throughout the water column and increased air-sea mixing at the surface. However, as gamete bundles are positively buoyant and float to the surface of the water upon release, the data obtained likely approximate the chemical conditions experienced by gametes during in situ fertilization.

**Fertilization Assays**

All fertilization experiments were conducted in field laboratories in Key Largo, FL. Montastraea faveolata 2008

On August 22, 2008, gametes from three *Montastraea faveolata* colonies were collected from Grecian Rocks, Key Largo, FL. Colonies spawned at approximately 23:30-23:40. Gamete bundles were taken to the boat and immediately diluted (1 part bundles to 3 parts filtered seawater). Gamete bundles disintegrated approximately 30-45 min after release, whereupon eggs and sperm from each colony were separated via pipet. Eggs were washed 5-7 times with filtered seawater to remove residual sperm. Equal volumes of eggs from each genet were combined to create a ‘stock’ egg batch for use in
fertilization experiments. The same was done with the sperm. Concentrated sperm representing equal contributions from three parental genotypes, was diluted in treatment seawater (435, 529, or 712 µatm) to obtain 1 part sperm to 199 parts treatment seawater (1:200). Only one sperm concentration was used for fertilization experiments.

Fertilization assays were conducted in pre-rinsed 6-well non-treated polystyrene tissue culture plates (BD Biosciences). Twelve ml of each sperm*pCO₂ combination were transferred via pipette into each of 18 wells. Twelve ml of (sperm-free) seawater was transferred into 6 wells of an extra plate as a negative (no-sperm) control. 10 µl of eggs were added to each well. Fertilization experiments commenced at approximately 03:30h, 4 h post-spawning. Experiments were conducted in a temperature-controlled room at ~28°C. Embryos were sub-sampled at 7 h and fixed in 10% formaldehyde. Subsequently, 100-200 undamaged eggs from each sub-sample were examined using a dissecting microscope and scored as either fertilized (showing normal cleavage patterns of cell division) or unfertilized (showing no signs of cleavage) (Figure 2.2). Percent fertilization data were arcsine transformed and analyzed using a Welch ANOVA.

![Figure 2.2](image-url)  
**Figure 2.2.** Fertilized (a) and unfertilized (b) *Montastraea faveolata* eggs. Unfertilized eggs are spherical and show no signs of cleavage.
Acropora palmata and Montastraea faveolata 2009

In 2009, the following methodology was applied to both *A. palmata* and *M. faveolata*. Samples from the *M. faveolata* experiments appeared to be contaminated (discussed below); therefore, only results for *A. palmata* are presented.

Stands of *A. palmata* were monitored for spawning on Elbow Reef and Sand Island, Upper Florida Keys on nights 2-6 following the full moon in August, 2009. On night 4 (August 10), colonies spawned at approximately 22:15h. Gamete bundles were collected from 2 previously genotyped genets at Elbow Reef and one at Sand Island. Gamete bundles disintegrated ~30 min after release, whereupon eggs and sperm from each colony were separated via pipet. Eggs were washed 5-7 times with filtered seawater to remove residual sperm. Equal volumes of eggs from each genet were combined to create a ‘stock’ egg batch for use in fertilization experiments. The same was done with the sperm.

Concentrated sperm ($3.21 \times 10^8$ sperm ml$^{-1}$), representing equal contributions from three parental genotypes, was diluted to $3.21 \times 10^6$, $1.61 \times 10^6$, $6.41 \times 10^5$, and $3.20 \times 10^5$ sperm ml$^{-1}$ in filtered treatment waters. Optimal sperm concentrations for other species of broadcast spawning corals have been reported to range between $10^5$-$10^6$ sperm ml$^{-1}$ (Oliver and Babcock 1992). One ml subsamples of each dilution were fixed in 10% formalin for verification of sperm concentrations (8 replicate counts by haemocytometer). Ten ml of each sperm dilution*treatment combination were transferred via pipette into each of 10 replicate 15 ml glass vials. Ten ml of (sperm-free) seawater were transferred into an extra vial as a negative (no-sperm) control. 100 µl of eggs (~200-250 eggs) were immediately added to each vial, and the mixture was swirled. Fertilization experiments
were initiated within 4 h of spawning, at 02:00h, and conducted at 28°C ± 0.1°C. Embryos were sub-sampled at 4 h and fixed in 10% formaldehyde. Subsequently, 100-200 undamaged eggs from each sub-sample were examined using a dissecting microscope and scored as either fertilized (showing normal cleavage patterns of cell division) or unfertilized (showing no signs of cleavage).

Nonlinear regressions (exponential rise to maximum, 2 parameter) were fit to percent fertilization at sperm concentration data. Regressions were fit separately for each CO$_2$ concentration using least squares residuals. The data were fit to the following model:

$$\%Fert = \text{max}\%Fert(1 - e^{-C \cdot SC})$$

where $\%Fert$ is the percent fertilization at sperm concentration $SC$, $\text{max}\%Fert$ is the asymptotic average maximum percent fertilization, and $C$ is a rate coefficient that determines how quickly (or slowly) the maximum is attained (i.e., the slope). Justification for the use of this model is provided below. A comparison of fit test was conducted to test the null hypothesis ($H_0$) that one curve best fit all data sets.

Data from the 0.32 x 10$^5$ sperm ml$^{-1}$ * 673 µatm treatment were omitted from the regression (‘x’ in Figure 2.9B), resulting in df = 30 at 673 µatm. Reliable data were not available for this cell, presumably as a result of sperm ‘contamination’ (i.e., a higher than targeted sperm concentration was likely added to this cell, resulting in higher than expected percent fertilization). This notion is supported by: 1) lack of the commonly documented trend of decreasing fertilization success with decreasing sperm concentration within the range of sperm concentrations used (Oliver & Babcock 1992; Willis et al. 1997; Levitan et al. 2004); and 2) the model results, which indicate an expected value
much less than that which was observed for that cell. Human error is the most likely source of additional sperm in this cell and may have resulted from error during the preparation of starting sperm dilutions or the addition of an incorrect dilution during the experiment. Additionally, one outlier was removed from the 998 μatm treatment, resulting in df=39 (‘+’ in Figure 2.9C); this outlier was greater than 2.5 SD away from the mean. Omitting these points did not change the statistical outcome of the regression analyses but provided a better fit for the model.

Model Justification

Coral fertilization success typically exhibits a non-linear response to sperm concentration, with fertilization success maximized at sperm concentrations ranging from $10^5$-$10^6$ sperm ml$^{-1}$ for at least 6 species of coral (Oliver & Babcock 1992; Willis et al. 1997; Levitan et al. 2004). Based on these prior studies, 4 sperm concentrations were selected within the range of $10^5$-$10^6$ sperm ml$^{-1}$ to evaluate the effect of elevated $p$CO$_2$ on coral fertilization success. The non-linear response of percent fertilization to sperm concentration observed in the present study is representative of previously published fertilization curves within the same range of sperm concentrations (Figure 2.3, black rectangle). The model selected, $\%Fert = \max\%Fert(1 - e^{-C*SC})$, approximates the relationship between fertilization and sperm concentration that has been previously documented in the literature and is one widely used in biological and physiological modeling where process-oriented events are measured relative to an asymptotic condition. In the present model, the asymptotic condition is the percent fertilization at high sperm concentrations. Conversely, at lower sperm concentrations the percent fertilization is non-linearly increasing towards the asymptote at a decreasing rate. The
proposed model reflects such a process, and the percent fertilization at sperm concentration data were, therefore, fit to nonlinear regressions (exponential rise to maximum, 2 parameter) as described in the Materials and Methods section.

Montastraea faveolata 2010

In 2010, fertilization experiments were repeated using gametes from *M. faveolata*. *Acropora palmata* did not spawn in August 2010. The fertilization experiments were conducted according to the previously outlined methodology (refer to methods for *A. palmata* 2009) with the following modifications: (1) a total of 8 sperm concentrations were used, ranging from $10^1$–$10^6$ sperm ml$^{-1}$ ($9.15 \times 10^6$, $9.15 \times 10^5$, $5.03 \times 10^5$, $9.15 \times 10^4$, $5.03 \times 10^4$, $9.15 \times 10^3$, $9.15 \times 10^2$, $9.15 \times 10^1$ sperm ml$^{-1}$); (2) two (as opposed to three) $p$CO$_2$ treatments were used, corresponding to 493 and 912 µatm; (3) 30 µl (as opposed to 100 µl) of eggs were added to each glass vial; (4) embryos were sub-sampled and fixed at 3 h (as opposed to 4 h); (5) percent fertilization data were fit to standard Gaussian distribution curves using least squared residuals. The three parameters of the Gaussian bell-shaped distribution curve are the AREA (area under the curve), mean, and SD; (6) Data from the $9.15 \times 10^4$ sperm ml$^{-1}$ * 800 µatm treatment were omitted from the regression (‘+’ in Figure 2.10). Reliable data were not available for this cell, presumably as a result of sperm contamination (discussed in *A. palmata* 2009). This notion is supported by the model results, which indicate an expected value much less than that which was observed for that cell.
Figure 2.3. Fertilization success as a function of sperm concentration (adapted from Oliver & Babcock 1992; Willis et al. 1997; Levitan et al. 2004). The black rectangle represents range of sperm concentrations identified as ‘optimal’ for fertilization success and the range of sperm concentrations utilized in 2009 fertilization experiments with *Acropora palmata*. *M. digitata* 1 and 2, *F. pentagona* and *P. sinensis* are from Oliver & Babcock 1992; *M. franksi* and *M. faveolata* are from Levitan et al. 2004; *A. latistella* is from Willis et al. 1997.

*Sperm Swimming Speeds*

*Acropora palmata* and *Montastraea faveolata* 2009

In 2009, sperm velocity was measured at each of the CO$_2$ levels (468, 673, 998 µatm) for both *A. palmata* and *M. faveolata*. Following the preparation of sperm concentration*CO$_2$* dilutions (described above), sperm from the two most concentrated dilutions were loaded into capillary slides and viewed on an IMT-2 inverted microscope at 40x. Sperm were videotaped using a Spot® Idea 3MP digital camera mounted to the microscope using a 0.55x coupler and connected to a computer. Spot® Advanced software was used to capture video files of swimming sperm. Each video was 30 seconds and captured at a frame rate of 33 frames second$^{-1}$. Three video files were captured per sperm concentration*CO$_2$* per species with the exception of the 1:100 dilution for *M.*
faveolata, when only one video file was captured per treatment. Video files were later imported to MetaMorph® Software for analysis of sperm velocities. Each spermatozoa was chosen by randomly selecting a frame number (between 1 and 903), then randomly selecting x and y coordinates corresponding to a point on the frame. All random points were generated using Microsoft Excel. The nearest motile sperm to the randomly generated coordinates was tracked for as long as possible (i.e., before leaving the field of view). On average, a single A. palmata spermatozoa was able to be tracked for 1.29 ± 0.04 (mean ± SEM) seconds before leaving the field of view; M. faveolata sperm swim faster and were only able to be tracked for 0.55 ± 0.01 seconds before leaving the field of view. A total of 20 individuals were tracked per video clip. D’Agostino & Pearson’s omnibus normality test and Bartlett’s test were used to verify the underlying assumptions of normality and homogeneity of variances, respectively. Data were analyzed using one-way ANOVAs.

Montastraea faveolata 2010

In 2010, A. palmata did not spawn in August. Montastraea faveolata sperm were collected, incubated at 3 different CO₂ levels according to the previously outlined methodology (see M. faveolata Fertilization Assays 2010), and transported to the University of Miami Miller School of Medicine’s Male Fertility Program of the Miami Project to Cure Paralysis. Sperm velocities were analyzed using an Integrated Visual Optical System Computer-Assisted Sperm Analyzer (IVOS, Hamilton Thorne). Due to limitations of the instrument (optimal concentrations of 2 x 10⁷ sperm ml⁻¹ for detection), sperm velocities were analyzed only for the most concentrated sperm dilution (9.15 x 10⁶
sperm ml$^{-1}$). Two trials were run for each CO$_2$ level. One trial was successful at $9.15 \times 10^5$ sperm ml$^{-1}$; however, few individuals (four to seven) were detected and results are, therefore, from a small sample size.

**Results**

*Carbonate Chemistry in Reef Waters*

Carbonate chemistry data for Elbow Reef and Grecian Rocks are presented in Figures 2.5 and 2.6, respectively. As several of these parameters are temperature-dependent (all except TA and DIC), sea-surface temperature (SST) over the course of the sampling period is presented in Figure 2.4. Temperature-dependent carbonate parameters were corrected for SST using CO2SYS software (E. Lewis, Brookhaven National Laboratory). Refer to Chapter 1, Carbonate Chemistry Analyses, for details regarding the dissociation constants used.

On Elbow Reef, maximum $p$CO$_2$ (minimum pH) was observed at ~09:00h, and minimum $p$CO$_2$ (maximum pH) was observed at ~21:30h. $p$CO$_2$ increased steadily over the course of the night from 21:30h-09:00h, presumably due to respiration. It then decreased throughout the day, presumably due to photosynthetic uptake. On Grecian Rocks, maximum $p$CO$_2$ (minimum pH) was observed at ~07:15h, and minimum $p$CO$_2$ (maximum pH) was observed at ~17:30h. $p$CO$_2$ increased steadily over the course of the evening/night, from 17:30h-07:15h and decreased throughout the day. This is consistent with what is expected from daily variation in surface temperature and the lag incurred by the release of CO$_2$ at the sea surface. Model parameters and regression coefficients for each parameter are listed on the respective graphs in Figures 2.5 and 2.6.
During coral spawning, gamete bundles break apart at the surface, releasing egg and sperm into the ocean. Bundles break apart starting ~30 min post-spawning. Coral sperm are typically viable for up to 3-4 h (Coll et al. 1994; D. Levitan, *pers. comm.*). Therefore, the window for fertilization is approximately 30-240 min post-spawning, with the highest chance of fertilization occurring immediately after bundles break apart. To characterize the water chemistry experienced by gametes during peak fertilization times, values for carbonate parameters were averaged during the 4 h post-spawning. Results are presented in Table 2.1. The average $pCO_2$ ($\mu$atm) during the 4 h post-spawning was 454 ± 12 at Elbow Reef and 458 ± 7 at Grecian Rocks. These values closely approximate those that were used as control values in fertilization experiments: 435 $\mu$atm in 2008, 468 $\mu$atm in 2009, and 493 $\mu$atm in 2010.
Figure 2.4. Sea surface temperature during 2009 water sampling at (a) Elbow Reef (sampled from 21:30h on August 21 to 19:30h on August 22) and (b) Grecian Rocks (sampled from 16:00h on August 22 to 15:00h on August 23). Daily averages are reported as mean ± SD.
Figure 2.5. Diurnal chemical conditions of surface seawater at Elbow Reef approximately 9 days post-spawning. Daily averages are reported as mean ± SD. Dashed black line represents mean value over the course of the sampling period.
Figure 2.6. Diurnal chemical conditions of surface seawater at Grecian Rocks approximately 8 days post-spawning. Daily averages are reported as mean ± SD. Dashed black line represents mean value over the course of the sampling period.
<table>
<thead>
<tr>
<th>Location</th>
<th>Date/Time</th>
<th>Salinity</th>
<th>T (°C)</th>
<th>TA (µmol kg⁻¹)</th>
<th>pH₉</th>
<th>pCO₂ (µatm)</th>
<th>HCO₃⁻ (µmol kg⁻¹)</th>
<th>CO₃²⁻ (µmol kg⁻¹)</th>
<th>CO₂ (µmol kg⁻¹)</th>
<th>TCO₂ (µmol kg⁻¹)</th>
<th>bCa²⁺ (mmol kg⁻¹)</th>
<th>Ωarag</th>
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<tbody>
<tr>
<td>Elbow Reef</td>
<td>Aug. 22, 2008</td>
<td>35.8 ± 0.1</td>
<td>29.3 ± 0.1</td>
<td>2360 ± 4</td>
<td>7.98 ± 0.01</td>
<td>489 ± 13</td>
<td>1819 ± 12</td>
<td>221 ± 3</td>
<td>12.4 ± 0.3</td>
<td>2052 ± 9</td>
<td>10.51 ± 0.03</td>
<td>3.56 ± 0.05</td>
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<td>Grecian Rocks</td>
<td>Aug. 23, 2008</td>
<td>35.8 ± 0.1</td>
<td>28.9 ± 0.1</td>
<td>2367 ± 19</td>
<td>7.94 ± 0.02</td>
<td>538 ± 30</td>
<td>1861 ± 27</td>
<td>207 ± 6</td>
<td>13.8 ± 0.8</td>
<td>2082 ± 24</td>
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<td>Aug. 21-22, 2009</td>
<td>36.5 ± 0.1</td>
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<td>8.00 ± 0.01</td>
<td>454 ± 12</td>
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<td>242 ± 4</td>
<td>11.3 ± 0.3</td>
<td>2046 ± 4</td>
<td>10.28 ± 0.03</td>
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<td>Grecian Rocks</td>
<td>Aug. 22-23, 2009</td>
<td>36.5 ± 0.1</td>
<td>30.32 ± 0.07</td>
<td>2375 ± 2</td>
<td>8.00 ± 0.01</td>
<td>458 ± 7</td>
<td>1786 ± 6</td>
<td>240 ± 2</td>
<td>11.3 ± 0.2</td>
<td>2037 ± 4</td>
<td>10.28 ± 0.03</td>
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<td>Elbow Reef</td>
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<td>2394 ± 4</td>
<td>8.00 ± 0.01</td>
<td>464 ± 10</td>
<td>1810 ± 9</td>
<td>240 ± 3</td>
<td>11.5 ± 0.3</td>
<td>2061 ± 7</td>
<td>9.69 ± 0.03</td>
<td>3.92 ± 0.05</td>
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<tr>
<td>White Banks</td>
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<td>32.5 ± 0.1</td>
<td>29.8 ± 0.1</td>
<td>2384 ± 4</td>
<td>8.00 ± 0.01</td>
<td>414 ± 3</td>
<td>1796 ± 6</td>
<td>243 ± 1</td>
<td>10.6 ± 0.1</td>
<td>2050 ± 5</td>
<td>9.56 ± 0.03</td>
<td>4.02 ± 0.01</td>
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Table 2.1. Physical and chemical conditions in situ near predicted spawning nights and times. Data from 2009 represent average conditions 4 h post-spawning time (N = 4 hourly samples, 22:00-2:00). See text for details. Data from 2008 and 2010 represent conditions near the predicted (Elbow Reef) and observed (Grecian Rocks and White Banks) spawning time (N = 1 or 2 samples). All measurements are based upon duplicate or triplicate analyses for each sampling period. pH₉, pCO₂, HCO₃⁻, CO₃²⁻, CO₂, TCO₂, and Ωarag were calculated using CO2SYS. Calcium concentration was calculated based on 10.28 mmol kg⁻¹ of Ca²⁺ at a salinity of 35 ppt.
**Fertilization Assays**

Chemical conditions during 2008-2010 fertilization experiments are given in Table 2.2. Control $p$CO$_2$ values were higher than 380 µatm because diurnal fluctuations in $p$CO$_2$ (due to photosynthetic uptake during the day and respiration at night) result in elevated $p$CO$_2$ values during evening hours when coral spawning and fertilization occur.

*Montastraea faveolata 2008*

The $p$CO$_2$ had a significant effect on fertilization success (Welch ANOVA, $F_{2,31.24}=58.54$, $P<0.0001$). Fertilization success was reduced by 25% at 529 µatm and 40% at 712 µatm respectively, compared to controls. Percent fertilization by treatment was as follows (mean ± SEM): 57 ± 1 (435 µatm); 43 ± 2 (529 µatm); 34 ± 2 (712 µatm). Cell counts using a haemocytometer in 2009 indicate that the sperm concentration used in this experiment was approximately $3 \times 10^6$ sperm ml$^{-1}$. In all treatments, a large number of fertilized embryos appeared to develop abnormally (Figure 2.7b). Oliver and Babcock (1992) and Coll et al. (1994) report negative effects of plastic containers on embryonic development and sperm longevity respectively. Subsequent experiments, conducted in 2009 and 2010, were restricted to glass vials (as opposed to 6-well plates), and the number of abnormal embryos decreased substantially.

*Acropora palmata 2009*

Fertilization success decreased as carbon dioxide concentration increased, with an average reduction of 12% and 13% (averaged across all sperm concentrations) at the high and mid CO$_2$ levels respectively. At the highest sperm concentration ($3.21 \times 10^6$ sperm ml$^{-1}$), fertilization was reduced by 7% and 12% at the mid and high CO$_2$ levels.
Figure 2.7. (a) Percent fertilization of *Montastraea faveolata* by $p$CO$_2$ treatment in 2008. (b) Abnormal development was observed in all treatments in 2008, presumably due to the use of plastic containers. All subsequent experiments (2009 and 2010) employed glass.

respectively. As sperm concentration declined, the reduction in fertilization success was exacerbated at elevated CO$_2$ levels: fertilization was reduced by 29% (mid CO$_2$) and 15% (high CO$_2$) at 1.61 x $10^6$ sperm ml$^{-1}$ and by 64% (mid CO$_2$) and 63% (high CO$_2$) at 6.41 x $10^5$ sperm ml$^{-1}$. At the most dilute sperm concentration (3.20 x $10^5$ sperm ml$^{-1}$), fertilization was reduced by 59% under the highest CO$_2$ treatment. Data from this sperm concentration were unreliable for the mid CO$_2$ treatment, presumably due to sperm contamination and were therefore omitted from the regression analysis (discussed in Materials and Methods, *Acropora palmata* Fertilization Assays 2009).

Nonlinear regressions were fit to percent fertilization at sperm concentration data (Figure 2.8); model parameters are listed in Table 2.3. These parameters are valid for *A. palmata* fertilization under laboratory conditions as described in the materials and methods and using sperm concentrations ranging from $10^5$-$10^6$ sperm ml$^{-1}$. A comparison of fit test rejected the null hypothesis that one curve best fit all data sets ($F_{4,103}=13.69$, $P < 0.0001$).
<table>
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<th>Salinity (°C)</th>
<th>T (µmol kg(^{-1}))</th>
<th>TA (µmol kg(^{-1}))</th>
<th>pH(_t)</th>
<th>pCO(_2) (µatm)</th>
<th>HCO(_3^-) (µmol kg(^{-1}))</th>
<th>CO(_2) (µmol kg(^{-1}))</th>
<th>CO(_2) (µmol kg(^{-1}))</th>
<th>TCO(_2) (µmol kg(^{-1}))</th>
<th>(^{b})Ca(^{2+}) (mmol kg(^{-1}))</th>
<th>(\Omega_{arag})</th>
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<tbody>
<tr>
<td>Montastraea faveolata 2008</td>
<td></td>
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<tr>
<td>Control</td>
<td>31.8 ± 0.1</td>
<td>28 ± 1</td>
<td>2335 ± 2</td>
<td>8.03 ± 0.01</td>
<td>443 ± 5</td>
<td>1839 ± 1</td>
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<td>Mid CO(_2)</td>
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<td>28 ± 1</td>
<td>2361 ± 4</td>
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<td>557 ± 11</td>
<td>1905 ± 6</td>
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<td>664 ± 5</td>
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<tr>
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</table>

Table 2.2. Physical and chemical conditions at the start of fertilization experiments (N=1 initial). All measurements are based upon duplicate or triplicate analyses for each sampling period. pH\(_t\), pCO\(_2\), HCO\(_3^-\), CO\(_2\), TCO\(_2\), and \(\Omega_{arag}\) were calculated using CO2SYS. Calcium concentration was calculated based on 10.28 mmol kg\(^{-1}\) of Ca\(^{2+}\) at a salinity of 35 ppt.
Data from *M. faveolata* assays indicate contamination such that data are not reliable. Percent fertilization was erratic both within and between treatments. No trend was observed of decreasing fertilization with decreasing sperm concentration. The source of potential contamination is unknown. Due to the unreliability of these data, results are not reported. Sperm velocities, however, are reported.

![Figure 2.8](image)

**Figure 2.8.** Non-linear regressions of 2009 *Acropora palmata* fertilization data by CO\textsubscript{2} treatment (mean ± SEM). Regressions were fit separately for each CO\textsubscript{2} concentration (refer to Figure 2.9). Parameter estimates are outlined in Table 2.3. Data from the 0.32 x 10\textsuperscript{5} sperm ml\textsuperscript{-1} * 673 \mu atm treatment were omitted from the regression due to the unavailability of reliable data for this cell (see Materials and Methods and Figure 2.9B).

**Table 2.3.** Parameter estimates for 2009 regressions of *Acropora palmata* fertilization.

<table>
<thead>
<tr>
<th></th>
<th>468 \mu atm</th>
<th>673 \mu atm</th>
<th>998 \mu atm</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Best-fit values</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Max % Fert</td>
<td>45.93</td>
<td>62.21</td>
<td>53.07</td>
</tr>
<tr>
<td>C</td>
<td>1.98</td>
<td>0.40</td>
<td>0.54</td>
</tr>
<tr>
<td><strong>Std. Error</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Max % Fert</td>
<td>3.30</td>
<td>17.97</td>
<td>6.02</td>
</tr>
<tr>
<td>C</td>
<td>0.46</td>
<td>0.20</td>
<td>0.12</td>
</tr>
<tr>
<td><strong>95% CI</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Max % Fert</td>
<td>39.25 to 52.62</td>
<td>25.39 to 99.01</td>
<td>40.86 to 65.28</td>
</tr>
<tr>
<td>C</td>
<td>1.06 to 2.90</td>
<td>-0.0003 to 0.80</td>
<td>0.30 to 0.78</td>
</tr>
<tr>
<td><strong>Degrees Freedom</strong></td>
<td>38</td>
<td>28</td>
<td>37</td>
</tr>
<tr>
<td><strong>R\textsuperscript{2}</strong></td>
<td>0.40</td>
<td>0.56</td>
<td>0.84</td>
</tr>
</tbody>
</table>
Figure 2.9. Individual non-linear regressions of 2009 *Acropora palmata* fertilization data for (a) 468 µatm (b) 673 µatm and (c) 998 µatm. Open symbols represent observed data; closed symbols represent expected data as determined by the model. ‘x’ in (b) represents the mean fertilization of the cell that was omitted when fitting the regression model (see Materials and Methods). ‘+’ in (c) represents an outlier that was omitted from the regression (see Materials and Methods). Regressions were fit separately for each CO₂ concentration. Parameter estimates are outlined in Table 2.3.
Montastraea faveolata 2010

Carbon dioxide ($p$CO$_2$) had a significant effect on fertilization success (Figure 2.10). A comparison of fit test rejected the null hypothesis that one curve best fit both sets of data ($F_{3,114} = 5.914, P<0.001$). As shown by model parameters, the mean value at 912 $\mu$atm is higher than that at 493 $\mu$atm, indicating that a higher sperm concentration is necessary to achieve maximum potential fertilization.

**Figure 2.10.** Results of 2010 *Montastraea faveolata* fertilization experiment (mean ± SEM). Non-linear regressions of fertilization data by CO$_2$ treatment. Regressions were fit separately for each CO$_2$ concentration. Parameter estimates are outlined in Table 2.4. Sperm concentrations were converted to log sperm ml$^{-1}$ for graphing purposes. Refer to text for actual sperm concentrations. Data from the $9.15 \times 10^4$ sperm ml$^{-1}$ * 912 $\mu$atm treatment (4.96 log sperm ml$^{-1}$) were omitted from the regression due to the unavailability of reliable data for this cell as a result of sperm contamination (refer to Materials and Methods). This point is indicated by a ‘+’.
Table 2.4. Parameter estimates for nonlinear regressions of *Montastraea faveolata* fertilization in 2010.

<table>
<thead>
<tr>
<th></th>
<th>493 µatm</th>
<th>912 µatm</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Best-fit values</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AREA</td>
<td>399.9</td>
<td>413.7</td>
</tr>
<tr>
<td>SD</td>
<td>1.610</td>
<td>1.758</td>
</tr>
<tr>
<td>Mean</td>
<td>6.530</td>
<td>7.154</td>
</tr>
<tr>
<td><strong>Std. Error</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AREA</td>
<td>41</td>
<td>99</td>
</tr>
<tr>
<td>SD</td>
<td>0.2</td>
<td>0.3</td>
</tr>
<tr>
<td>Mean</td>
<td>0.2</td>
<td>0.5</td>
</tr>
<tr>
<td><strong>95% CI</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AREA</td>
<td>318 to 482</td>
<td>215 to 613</td>
</tr>
<tr>
<td>SD</td>
<td>1.3 to 1.9</td>
<td>1.1 to 2.4</td>
</tr>
<tr>
<td>Mean</td>
<td>6.2 to 6.9</td>
<td>6.2 to 8.1</td>
</tr>
<tr>
<td><strong>Degrees Freedom</strong></td>
<td>61</td>
<td>53</td>
</tr>
<tr>
<td><strong>R²</strong></td>
<td>0.84</td>
<td>0.79</td>
</tr>
</tbody>
</table>

_Sperm Swimming Speeds_

Acropora palmata and Montastraea faveolata 2009

In 2009, there was no consistent significant effect of $pCO_2$ on sperm velocity at the two sperm concentrations that were analyzed for either *A. palmata* or *M. faveolata* (Figures 2.11 and 2.12). Sperm velocities of *A. palmata* ranged from 77-246 µm sec$^{-1}$. Sperm velocities of *M. faveolata* ranged from 197-434 µm sec$^{-1}$. Morita et al. (2006) reported *A. digitifera* sperm velocities to range from 200-350 µm sec$^{-1}$. The marginally lower sperm velocities reported here may be due to: a) species-specific differences; b) slight temperature differences (though both experiments were conducted at 28°C); or c) gamete aging – filming in the present study commenced ~3 h post-spawning, whereas gametes in Morita et al. (2006) were collected from colonies in the laboratory and presumably used within a shorter period of time.
With respect to *A. palmata* data, there was no *a priori* reason to believe that the effect of CO$_2$ on fertilization success would be dependent on sperm concentration (refer to Results of 2009 *A. palmata* fertilization experiments). Due to time restrictions (aging of gametes), it was not feasible to videotape all sperm concentrations. The two most concentrated dilutions were chosen to maximize the number of individuals that could be analyzed. While velocities are reported for these two concentrations, results of the fertilization assays revealed a concentration-dependent treatment effect such that the effect of CO$_2$ on fertilization success was exacerbated at lower sperm concentrations (those at which sperm were not videotaped). At the two concentrations that were videotaped, CO$_2$ had little or no effect on fertilization success. In the two sperm concentrations that were analyzed, only one significant difference was observed in fertilization success (468 µatm and 673 µatm at 1.61 x 10$^6$ sperm ml$^{-1}$, F$_{2,27}$=3.579, $P<0.05$, Figure 2.11E). This trend was also observed in trial 2 of the associated sperm assays (Figure 2.11F), but the difference was not consistently observed (i.e., absent from Trials 1 and 3). It would have been ideal to relate reductions in sperm velocities to reductions in fertilization success, but this would have required data from the lowest sperm concentrations. The original intent was to repeat sperm analyses in 2010, focusing on the more dilute sperm concentrations; however, *A. palmata* did not spawn in August, 2010. Future studies should attempt to analyze sperm motility/velocity across a range of sperm concentrations to relate changes to fertilization success.
Figure 2.11. (a,b) Scanning electron micrographs of *Acropora palmata* spermatozoa. Scale bars for each view are indicated on SEM micrographs. (c,e) Percent fertilization and (d,f) sperm swimming speeds (mean ± SEM) of *A. palmata* from the two most concentrated sperm dilutions in 2009, (c,d) 3.21 x 10^6 sperm ml^-1 and (e,f) 1.61 x 10^6 sperm ml^-1. Three trials were conducted at each sperm concentration. Asterisks indicate significant differences.
Figure 2.12. (a,b) Scanning electron micrographs of Montastraea faveolata spermatozoa. Scale bars for each view are indicated on SEM micrographs. (c,d) sperm swimming speeds (mean ± SEM) of M. faveolata from the two most concentrated sperm dilutions in 2009, (c) 8.45 x 10^6 sperm ml⁻¹ and d) 3.12 x 10^6 sperm ml⁻¹. Only one trial was conducted at 8.45 x 10^6 sperm ml⁻¹. Three trials were conducted at 3.12 x 10^6 sperm ml⁻¹. Asterisks indicate significant differences between treatments. Percent fertilization for each dilution is not available due to sample contamination.

Montastraea faveolata 2010

Velocity data for Montastraea faveolata sperm are presented in Table 2.5. Sperm velocities ranged from 25-252 µm sec⁻¹. Average area of M. faveolata sperm heads was estimated to be 1.4 ± 0.9 µm².
Table 2.5. Data acquired for *Montastraea faveolata* sperm motility and velocity in 2010 using the IVOS Computer-Assisted Sperm Analyzer. Velocity reported is ‘Track Velocity’ (VCL). Data were collected ~3-4 h. post-spawning. Asterisks (*) indicate erroneous motility – instances for which the system was only able to detect a small number of individuals (4-7); motility is, therefore, based on a small sample size and may be unreliable.

<table>
<thead>
<tr>
<th>Total Count</th>
<th>Motile Count</th>
<th>Rapid Count</th>
<th>Medium Count</th>
<th>Slow Count</th>
<th>Static Count</th>
<th>Motility (%)</th>
<th>Velocity (µm s⁻¹)</th>
<th>T (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Trial 1 - 9.15 x 10⁶ sperm ml⁻¹</strong></td>
<td></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>493 µatm</td>
<td>220</td>
<td>202</td>
<td>192</td>
<td>10</td>
<td>15 (7%)</td>
<td>3</td>
<td>92%</td>
<td>252</td>
</tr>
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<td></td>
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<td></td>
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</tr>
<tr>
<td>912 µatm</td>
<td>153</td>
<td>110</td>
<td>94</td>
<td>16</td>
<td>28</td>
<td>15</td>
<td>72%</td>
<td>119</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td><strong>Trial 2 - 9.15 x 10⁶ sperm ml⁻¹</strong></td>
<td></td>
<td></td>
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<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>493 µatm</td>
<td>121</td>
<td>106</td>
<td>104</td>
<td>2</td>
<td>10</td>
<td>5</td>
<td>88%</td>
<td>226</td>
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</tr>
<tr>
<td>912 µatm</td>
<td>253</td>
<td>234</td>
<td>218</td>
<td>16</td>
<td>16</td>
<td>3</td>
<td>92%</td>
<td>188</td>
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<td></td>
<td></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td><strong>Trial 1 - 9.15 x 10⁵ sperm ml⁻¹</strong></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>493 µatm</td>
<td>4</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>*75%</td>
<td>99.7</td>
</tr>
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<td></td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>912 µatm</td>
<td>7</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>4</td>
<td>0</td>
<td>*43%</td>
<td>24.5</td>
</tr>
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</tr>
</tbody>
</table>

Discussion

Fertilization experiments, conducted with both *A. palmata* and *M. faveolata*, indicate that $p$CO$_2$ significantly affects fertilization rate. In both species, the effect of $p$CO$_2$ was dependent on the sperm concentration. At the highest sperm concentrations (approaching optimal concentrations but unlikely to occur in nature), little to no treatment
effect was observed; as sperm concentration declined, the effect of $pCO_2$ was exacerbated. No reduction in maximum potential fertilization was observed; however, higher sperm concentrations were necessary to achieve high fertilization success. For *A. palmata*, the two elevated CO$_2$ treatments were not significantly different from each other. These results suggest that there may be a threshold response to elevated CO$_2$ within the range of CO$_2$ values and sperm concentrations that were tested for this coral species.

Sperm swimming speed data collected in 2009 for *A. palmata* and *M. faveolata* were inconclusive with respect to the effect of $pCO_2$. However, preliminary results from the IVOS system using *M. faveolata* sperm in 2010 indicate an effect of $pCO_2$ on sperm motility and swimming speed. Refer to Chapter 5 for further discussion of fertilization and sperm swimming speed results.
Chapter 3: Effect of Ocean Acidification on Settlement Processes

Summary

To evaluate the effects of ocean acidification (OA) on larval settlement, pre-settlement physiology and settlement behavior of coral larvae were tested at three $pCO_2$ levels: ambient seawater (380 µatm) and two $pCO_2$ scenarios that are projected to occur by the middle (560 µatm) and end (800 µatm) of the century. Larval physiology was evaluated by measuring respiration rates and C/N ratios of the common Caribbean coral *Porites astreoides*. Settlement behavior was evaluated by testing direct (physiological disruption of metamorphosis) and indirect (interference with settlement via benthic habitat cues) effects of $pCO_2$ on the successful settlement and metamorphosis of planulae of two common Caribbean coral species, *P. astreoides* (brooder) and *Acropora palmata* (spawner). Metabolism of *P. astreoides* larvae was depressed by 27% and 63% at 560 and 800 µatm, respectively, compared with controls. Results of C/N analyses were inconclusive. Settlement of both *P. astreoides* and *A. palmata* was reduced at elevated $pCO_2$; results indicate that ocean acidification primarily affects settlement via indirect pathways, whereby acidified seawater alters the substrate community composition, limiting the availability of settlement cues.

Background

For corals, the transition from a planktonic planula larva to a sessile benthic juvenile involves two steps - settlement and metamorphosis. Settlement can be defined as “the behavioral response of a larva when it stops dispersal and selects substrate for recruitment” while metamorphosis refers to “the subsequent morphological and
physiological changes that pelagic larvae undergo to become benthic juveniles” (Ritson-Williams 2009, p. 443). These are the definitions that will be used here. Settlement can be influenced by the conditions the larvae experience in the plankton, or by positive or negative cues on the benthos or in the overlying water-column (Ritson-Williams et al. 2009). During the developmental and dispersal phase, planktonic larvae are exposed to water quality conditions that can affect their health, behavior, and settlement (Vermeij et al. 2006; Ritson-Williams et al. 2009). Acute exposure to environmental stressors may affect the larval condition, resulting in delayed or altered metamorphosis and/or carry-over/latent effects on later life history stages including reduced fitness, decreased growth rate and/or fecundity, etc. (Pechenik 2006). Vermeij et al. (2006) showed that altering planktonic conditions, namely salinity, affected settlement performance of *Montastraea faveolata* larvae, which translated into greater pre- and post-settlement mortality.

In addition to planktonic conditions, substrate community composition plays a critical role in determining coral larval settlement. Coral planulae do not settle at random; larvae of many coral species actively select a site of permanent attachment using external chemical cues that induce metamorphosis (Morse et al. 1988, 1994, 1996; Heyward & Negri 1999). Both positive settlement cues from crustose coralline algae (CCA) and/or microbial biofilms and settlement interference by turf algae have been previously documented (Morse et al. 1988; Webster et al. 2004; Birrell et al. 2005; Kuffner et al. 2006; Vermeij & Sandin 2008; Ritson-Williams et al. 2010). Environmental factors that alter or interfere with these cues have the potential to greatly impact coral recruitment and future population dynamics.
To determine whether near-future OA scenarios threaten the successful settlement and metamorphosis of coral larvae, pre-settlement physiology and settlement behavior of coral larvae were tested at three $p$CO$_2$ levels: ambient seawater (380 µatm) and two $p$CO$_2$ scenarios that are projected to occur by the middle (560 µatm) and end (800 µatm) of the century. Larval physiology was evaluated by measuring respiration rates and C/N ratios of the common Caribbean coral *Porites astreoides*. To evaluate the effects of OA on settlement and metamorphosis, planulae of two common Caribbean coral species, *P. astreoides* (brooder) and *Acropora palmata* (spawner) were settled in either ambient or acidified seawater in 2007-2009. Distinctions are made between direct (physiological disruption of metamorphosis) and indirect (interference with settlement via benthic habitat cues) effects. By conditioning tiles in ambient seawater and settling larvae onto those tiles in treatment seawater, the potential for acidification to directly impair larval settlement and metamorphosis was assessed. Alternatively, by conditioning tiles in treatment seawater and settling larvae in treatment seawater the potential for OA to indirectly affect settlement by altering the substrate community composition and the availability of biological and chemical settlement cues was assessed. Point-count and spectrofluorometry analyses were used to quantify differences in substrate community composition of substrata conditioned at different $p$CO$_2$ levels.
Materials and Methods

Collection of larvae

Porites astreoides

Porites astreoides is a brooding coral that spawns predictably near the new moon from April through June (McGuire 1998), rendering larvae easy to collect for use in laboratory experiments. In 2007, adult colonies were collected from The Rocks, an inshore patch reef near Tavernier, Florida (FL, USA), several days prior to the new moon in May and June and maintained in a closed re-circulating seawater system for approximately 1 week during the predicted period of larval release. Colonies were stored in mesh-lined containers during the nights of release. Following release, larvae were transferred to sterile containers with filtered seawater and transported to the University of Miami’s Rosenstiel School of Marine and Atmospheric Science (RSMAS). Approximately 700 larvae were collected in May and 400 in June.

In 2008 and 2009, 12 adult colonies of P. astreoides were collected from Little Grecian, an offshore bank-barrier reef near Key Largo, Florida (USA), several days prior to the new moon in May and June (2008) and April and May (2009). Colonies were maintained in a flow-through seawater system at the Rosenstiel School for approximately 1 week during the predicted period of larval release. Larvae were collected according to the methods outlined by Kuffner et al. (2006). On the mornings following release, larvae from each parent colony were pooled and transferred to sterile containers with filtered seawater for use in experiments.

In May 2010, inclement weather prevented the direct collection of coral colonies and larvae. Therefore, larvae (~ 800) were obtained from eight P. astreoides colonies
(~100 larvae from each of 8 colonies), collected by a team of researchers (Smithsonian Marine Station, Fort Pierce) from two shallow (5-6 m) patch reefs near Summerland Key, FL. Colonies were maintained at the Mote Marine Laboratory in Summerland Key during the period of larval release, and larvae were pooled upon release for use in experiments.

Acropora palmata

*Acropora palmata* gametes were collected according to the methodology outlined in Chapter 2. Larvae were reared to competency for use in settlement experiments.

**CHNS-O Analyses**

As coral larvae are lecithotrophic (defined here as containing egg-derived yolk, on which they feed), the lipid content or change in lipid content is indicative of energy metabolism. The C/N ratio is considered a good proxy for an organism’s condition, as it reflects the ratio of lipids and carbohydrates to proteins (Bodin et al. 2007). Additionally, a strong correlation between the percentage of lipids (dry tissue weight) and C/N ratio is reported for a range of marine and terrestrial animals (Post et al. 2007). Consumption of stored energy in the form of lipids is expressed as a decrease in the C/N ratio (Alamaru et al. 2009). The stress response exhibited by coral larvae in response to $pCO_2$ exposure may alter energy metabolism, thereby affecting the lipid reserves. To compare the lipid content of larvae and determine if $pCO_2$ exposure results in altered energy metabolism, free-swimming *P. astreoides* planulae were collected immediately following release and allowed to develop in treatment water for 48 h in 2008, 24 h in 2009, 80 h in 2010. A known number of larvae from each treatment were sampled from batch cultures and
transferred to Whatman GFA filters. Filters were dried to a constant weight. Oven-dried larval tissue was combusted (1,025 °C) and analyzed in a CE Elantech elemental analyzer (Model 1106, CHNS-O). 2,5-Bis(5-tert-butylbenzoxazol-2-yl) thiophene BBOT, dl-Methionine, Sulphanilamide, and L-Cystine were used as standards. All samples were blank corrected. Carbon and nitrogen data were used to create mean C/N for each pCO₂ treatment at each time interval.

**Larval Metabolism**

In May 2010, larval metabolic rates were measured twice at each of the three CO₂ levels. The first experiment was conducted ~24 h after spawning (AS), and the second experiment was conducted ~48 h AS. Each respiration experiment involved four chambers (run simultaneously): three contained filtered treatment water (0.2 µm; ambient, mid CO₂, or high CO₂) and 20 larvae; the fourth chamber contained filtered ambient seawater and no larvae and was used to correct for background respiration rates. A preliminary experiment was conducted using different numbers of larvae in each chamber (5, 10, 20, or 30) to determine the optimal number for the subsequent experiments. Prior to the experiments, the 4 chambers were run with filtered seawater alone to ensure that they were reading uniformly; chambers were calibrated in air-bubbled filtered seawater at the measurement temperature (26°C), and a saturated oxygen value was obtained by computation of the saturation concentration (Benson & Krause 1984).

Chambers and larvae were dark-acclimated for 2 h prior to the start of each experiment, and experiments were conducted in a darkened, constant-temperature water
bath maintained at 26°C. Respiration was measured over a 2 h interval as oxygen flux using YSI 5750 oxygen electrodes, connected to an ENDECO 1125 4-channel Pulsed DO Sensor. A PC computer was used to log the temperature and oxygen data output every 10 min from each of the 4 oxygen electrodes. The oxygen consumption rate was determined by regressing oxygen concentration against time. The oxygen consumption rate determined in each chamber was corrected for the background consumption rate in the control chamber, multiplied by the volume of water in the chamber (~20 ml), and divided by the number of larvae (20) to obtain the respiration rate in nmoles of oxygen larva\(^{-1}\) h\(^{-1}\). A total of 6 independent estimates of larval respiration rate were obtained.

The first incubation commenced at 14:00h (24 h AS), with CO\(_2\) levels close to the target levels of 380, 560 and 800 µatm. At 48 h AS, the incubation commenced ~2 hours earlier in the day, at 12:00h. Due to natural diurnal variation in the seawater system (resulting from photosynthetic uptake of CO\(_2\) throughout the day), the ambient and mid-CO\(_2\) levels were slightly higher at 48 h AS than 24 h AS. The same should have been true for the high CO\(_2\) treatment, but a blocked airstone resulted in a lower than target CO\(_2\) level. Due to both the natural diurnal variability and the airstone blockage, the CO\(_2\) levels varied between the two experiments and averaging values from the two experiments for analysis of variance was deemed inappropriate. Rather, CO\(_2\) was treated as a continuous variable and data from both experiments were analyzed by linear regression analysis using least squared residuals.
Settlement Experiments

Porites astreoides 2007

Seawater chemistry was manipulated via HCl additions, as described in Chapter 1, to obtain one of three aragonite saturation states: \( \Omega_{\text{arag}}=3.2 \) (control); \( \Omega_{\text{arag}}=2.6 \) (mid); and \( \Omega_{\text{arag}}=2.2 \) (low). Settlement experiments were conducted in 300 ml plastic Solo\textsuperscript{©} cups, maintained in a water bath at a constant temperature of 25°C. Each cup contained three 1 cm\(^2\) limestone tiles that were pre-conditioned in situ for approximately 1 month. Tiles were nested in clean, baked silica sand to force larvae to settle on the flat, upper surface of the tile, ensuring accurate growth measurements. Silica sand was used to avoid the potential buffering effects of limestone sediments. Cups were randomly assigned to treatments, 250 ml of treatment water and a known number of larvae were added to each cup, and larvae were allowed 1 week to settle. Settlement cups were un-aerated and tightly covered with a sheet of Plexiglas to prevent gas exchange. Water was exchanged every 48 h taking care not to disturb larvae. In May, 12 settlement cups were prepared for each treatment, with 20 larvae introduced into each cup. In June, 8 cups were used, with 15 larvae per cup. Settlement was confirmed by examining juveniles under a dissecting microscope.

Porites astreoides 2008-2009

In 2008, two settlement experiments were conducted simultaneously. In the first experiment, limestone settlement tiles were pre-conditioned in ambient seawater (380 \( \mu \)atm), and larvae were settled onto the tiles in treatment seawater (380, 560, or 800 \( \mu \)atm). In the second experiment, settlement tiles were pre-conditioned in treatment
seawater, and larvae were settled in treatment seawater (corresponding to the treatment in which the tiles were conditioned). Details of the tile-conditioning and settlement assays are provided below.

Seawater chemistry was manipulated via direct bubbling with carbon dioxide-enriched air, as described in Chapter 1. To verify distinct treatments, water samples were taken at the start and end of settlement experiments; samples were taken weekly during tile conditioning. Chemical and physical conditions that persisted during each experiment are outlined in Tables 3.7 and 3.8.

Prior to settlement assays, commercially-sourced limestone tiles were preconditioned for 40 days in flow-through aquaria with either ambient seawater (380 µatm) or treatment seawater (560 or 800 µatm). Mean tile dimensions were 20.6 ± 0.1 mm x 12.0 ± 0.1 mm x 3.23 ± 0.06 mm (mean ± SEM), and average tile mass was 1.89 ± 0.04 grams. A single source of live rock was divided equally amongst the aquaria to provide a consistent source of crustose coralline algae and microfauna.

Settlement assays were conducted in pre-rinsed 6-well non-treated polystyrene tissue culture plates (BD Biosciences). One settlement tile, 10 ml of treatment water and 10 larvae (2 days old) were randomly added to each well. Plates were securely covered and submerged in treatment tanks to ensure temperature control (28°C) and prevent gas exchange. Sixteen wells were used per treatment. Tiles were examined after 24 h. The number of settled larvae on the top, bottom and sides of each tile was counted using a dissecting microscope. Larvae were scored as “settled” when they had fully metamorphosed (flat/disc-shaped appearance rather than pear-shaped), with little or no possibility of active detachment and further migration (Harrison & Wallace 1990). Wells
in which all ten larvae could not be accounted for at the end of the experiment were eliminated from the statistical analysis, resulting in the following sample sizes: Ambient Tile Experiment: N=15 (380 µatm); N=16 (560 µatm); N=14 (800 µatm) and Treatment Tile Experiment: N=15 (380 µatm); N=13 (560 µatm); N=14 (800 µatm). Percentage data were arcsine transformed and analyzed using one-way ANOVAs. D’Agostino & Pearson omnibus test and Levene’s test were used to verify the underlying assumptions of normality and homogeneity of variances, respectively. Where significant differences were detected, post-hoc HSD Tukey analyses were used to determine which treatments differed from each other.

In 2009, the second experiment (Treatment Tiles) was repeated according to the previously outlined methodology with the following modifications: 30 wells were used per treatment (with similar omissions when all larvae were not accounted for), resulting in the following sample sizes: N=30 (380 µatm); N=30 (560 µatm); N=29 (800 µatm). Experiments were conducted at 26°C. Percentage data were arcsine transformed and analyzed using a Kruskal-Wallis test.

Data from both Treatment Tile Experiments (2008 and 2009) were pooled and analyzed by linear regression analysis using least squared residuals.

Acropora palmata 2009

In 2009, Acropora palmata gametes that were not used for fertilization experiments were fertilized, reared to competency in ambient seawater, and used in assays to test the effects of pCO₂ on settlement success (refer to Chapter 2 for details of gamete collection and fertilization). Five-day old larvae (5 dAS) were introduced to settlement assays
where they were offered limestone settlement tiles that had been pre-conditioned in re-circulating aquaria corresponding to the three CO$_2$ treatments for 40 d prior. A single source of live rock was divided equally amongst the 3 treatment aquaria to provide a consistent source of crustose coralline algae and microfauna during the conditioning phase of the experiment. Settlement assays were conducted in 6-well non-treated polystyrene tissue culture plates (BD Biosciences) using the same treatment water as was used for substrate conditioning. One settlement tile, 10 ml of treatment water and 10 larvae were randomly added to each well. Four plates (24 wells) were used per treatment; plates were securely covered and submerged in treatment tanks to ensure temperature control (28°C) and prevent gas exchange (equilibration of mid and high CO$_2$ with atmosphere). Water was exchanged by syringe every 48 h taking care not to disturb larvae.

Tiles were examined at 6 d, 8 d, and 11 dAS. Prior studies have shown that the first permanent larval settlement (i.e., metamorphosed and permanently settled juvenile polyps) of two acroporid species occurred at 5–6 dAS, and most larval settlement (85–97% of total) occurred within 9–10 dAS (Nozawa & Harrison 2008). The number of settled larvae on the top and bottom of each tile was counted at 11 dAS using a dissecting microscope. Percentage data were arcsine transformed and analyzed using a one-way ANOVA. D’Agostino & Pearson omnibus test and Levene’s test were used to verify the underlying assumptions of normality and homogeneity of variances, respectively.
Substrate Community Composition Analyses

Point Count Analyses 2008

Having observed no direct effect of \( pCO_2 \) on settlement success in 2007, the following year settlement substrates were conditioned in ambient (380 \( \mu \text{atm} \)) and elevated \( pCO_2 \) (800 \( \mu \text{atm} \)) seawater for 45 d to determine if \( pCO_2 \) affects substrate community composition. Ten tiles were conditioned per treatment. After 45 d, tiles were removed from treatment conditions and the surfaces of the tiles were photographed using a camera mounted on a dissecting scope. To quantify differences in percent cover by crustose coralline algae (a red alga that induces settlement of some coral species), Coral Point Count with Excel extensions (CPCe) Software was used to randomly generate 50 points per photograph (tile). The substratum underlying each random point was classified as CCA or non-CCA. Each tile was analyzed 10 times (each time generating 50 new, random points), and data were averaged to produce a mean percent cover of CCA per tile. Percent data were arcsine transformed and analyzed using a t-test.

Spectrofluorometry Analyses

Porites astreoides 2009

To determine whether conditioning settlement substrates at the different \( pCO_2 \) levels altered the epilithic algal communities (and the availability of potential settlement cues), pre-conditioned tiles that were not used in \( P. \ astreoides \) settlement assays in 2009 (but conditioned simultaneously) were placed in 15 ml tubes and immediately frozen for use in spectrofluorometry analyses. Epilithic algal communities were extracted from tiles, and concentrations of Chlorophylls \( a, b, c \), phycoerythrin and phycocyanin were
determined by measuring the fluorescent emission of the pigments extracted from the settlement tiles using a SPEX Fluorolog-3 spectrofluorometer. Pigments were extracted using a solution of 10 ml dimethyl sulfoxide (DMSO) and 15 ml 90% acetone for chlorophyll (chl) analyses; DMSO was added 30 min prior to the addition of acetone. 10 ml phosphate buffer (0.05M H₂KPO₄, 0.05M HK₂PO₄, 0.01% mercaptoethanol, pH 6.5) was used for the extraction of phycoerythrin (PE) and phycocyanin (PC). Pigment extractions took place overnight. Ten tiles were sampled per treatment (380, 560, 800 µatm) per analysis (PE/PC or chl). Pigment concentrations were normalized to the weight of the tile. Emission peaks (wavelengths) for each pigment are as follows: chl a - 670 nm; chl c - 635 nm; chl b - 650 nm; PE - 570 nm; PC - 640 nm.

PE/PC data were square root transformed to meet assumptions of homogeneity of variances, and all data were analyzed using one-way ANOVAs. Where significant differences were detected, post-hoc HSD Tukey analyses were used to determine which treatments differed from each other.

Acropora palmata 2009

To determine whether the observed differences in settlement were due to differences in the epilithic algal communities of settlement substrates conditioned at different pCO₂ levels, biofilms of pre-conditioned tiles that were not used in A. palmata settlement assays were sampled at the time of settlement and analyzed using spectrofluorometry, accordingly to the previously outlined methodology. PE/PC data were log transformed to meet assumptions of normality. Data were analyzed using one-way ANOVAs. Remaining tiles were left to continue conditioning and sampled again at
69 days (29 days after settlement). At 69 d, no tiles remained for chl analyses in the mid CO₂ treatment (560 µatm).

Results

CHNS-O Analyses

In 2008, the baseline C/N of larvae immediately following release from parent colonies was 10.5 ± 0.1 (N=10) (mean ± SEM). pCO₂ had a significant effect on C/N following the 48 h incubation (F₂,26 = 31.76, P<0.0001), with final C/N as follows: 9.9 ± 0.3 (N=9) (380 µatm); 8.6 ± 0.2 (N=10) (560 µatm); 11.4 ± 0.2 (N=10) (800 µatm). C/N of control larvae did not significantly decrease over 48 h. However, C/N of larvae incubated at 560 µatm decreased significantly, and C/N of larvae incubated at 800 µatm increased significantly (Figure 3.1).

In 2009, baseline measurements were not collected at the time of larval release. However, pCO₂ had a significant effect on C/N following the 24 h incubation (F₂,26 = 71.40, P<0.0001), with final C/N as follows (mean ± SEM): 8.9 ± 0.1 (N=10) (380 µatm), 10.6 ± 0.1 (N=10) (560 µatm); 10.9 ± 0.1 (N=10) (800 µatm) (Figure 3.2).

In 2010, the baseline C/N of larvae immediately following release from parent colonies was 9.4 ± 0.2 (N=10) (mean ± SEM). Larvae were incubated in treatment water and sampled at 20 h, 36 h, 60 h, and 82 h. No larvae remained in the mid CO₂ treatment (560 µatm) by 82 h. Analysis through time is of limited utility as inconsistencies in the time of day at which samples were taken may have resulted in large amounts of variation in carbon and nitrogen levels (since P. astreoides larvae contain zooxanthellae, carbon stores likely fluctuate throughout the day as a result of photosynthesis). However,
comparative analyses at each discrete time point remain valid. While the trends observed at each individual time-point are comparable to those observed after 48 h in 2008 (depressed C/N at mid CO₂ and elevated C/N at high CO₂), the only significant difference in 2010 was at 36 h – when larvae at 800 µatm had significantly higher C/N than larvae at 560 µatm (Figure 3.3). The lack of significance is likely due to low sample size during this year (ranging from 4-7, depending on the treatment and sampling period). Inclement weather prevented the direct collection of coral colonies in 2010, and experiments depended on ‘leftover’ larvae from a research crew at the Smithsonian Marine Institute. The overall larval supply and the number of replicates that could be run was therefore limited.

**Larval Metabolism**

Chemical conditions that persisted during the respiration incubations are presented in Table 3.3. Larval metabolic rates decreased significantly with increasing $pCO_2$ ($F_{1,4}=32.74, \ P<0.005$) (Figure 3.4). Model parameters obtained from regression analysis indicate a 27% and 63% reduction in metabolic rates at $pCO_2$ levels that are projected to occur by the middle (560 µatm) and end (800 µatm) of this century. Initial O₂ concentrations in each experiment and treatment were close to 200 µmol kg⁻¹ (the expected saturation concentration at 26°C and 35 ppt), and absolute O₂ concentrations never fell below 160 µmol kg⁻¹, 80% of saturation (2 mg l⁻¹ or ~60 µmol kg⁻¹ is typically
**Figure 3.1.** C/N of *Porites astreoides* larvae following a 48 h incubation in treatment water at 28°C in 2008. Dashed line represents baseline C/N, determined from samples taken at 0 h (i.e., immediately after release from parent colony and prior to introduction to treatment conditions). Sample sizes were as follows: N=10 (0 h); N=9 (318 µatm); N=10 (565 µatm); N=10 (914 µatm).

**Table 3.1.** Analysis of variance table and pair-wise multiple comparisons of C/N 48 h after incubation of *Porites astreoides* larvae in treatment water in 2008.

<table>
<thead>
<tr>
<th>ANOVA</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>F Ratio</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>2</td>
<td>40.29</td>
<td>20.14</td>
<td>31.76</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Residual</td>
<td>26</td>
<td>16.49</td>
<td>0.6342</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>28</td>
<td>56.78</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Tukey’s HSD</th>
<th>Mean Diff.</th>
<th>Q</th>
<th>(P&lt;0.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td>318 v 565 µatm</td>
<td>1.253</td>
<td>4.844</td>
<td>Y</td>
</tr>
<tr>
<td>318 v 914 µatm</td>
<td>-1.579</td>
<td>6.104</td>
<td>Y</td>
</tr>
<tr>
<td>565 v 914 µatm</td>
<td>-2.833</td>
<td>11.25</td>
<td>Y</td>
</tr>
</tbody>
</table>
Figure 3.2. C/N of *Porites astreoides* larvae following a 24 h incubation in treatment water at 26°C in 2009. Baseline samples were not taken this year. N=10 in all treatments.

Table 3.2. Analysis of variance table and pair-wise multiple comparisons of C/N 24 h after incubation of *Porites astreoides* larvae in treatment water in 2009.

<table>
<thead>
<tr>
<th>ANOVA</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>F Ratio</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
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<td>21.80</td>
<td>10.90</td>
<td>71.40</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Residual</td>
<td>27</td>
<td>4.122</td>
<td>0.1527</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>29</td>
<td>25.92</td>
<td></td>
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</tbody>
</table>

Tukey’s HSD

<table>
<thead>
<tr>
<th>Mean Diff.</th>
<th>Q</th>
<th>(P&lt;0.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td>345 v 563 µatm</td>
<td>-1.659</td>
<td>13.43</td>
</tr>
<tr>
<td>345 v 843 µatm</td>
<td>-1.927</td>
<td>15.60</td>
</tr>
<tr>
<td>563 v 843 µatm</td>
<td>-0.2678</td>
<td>2.167</td>
</tr>
</tbody>
</table>
Figure 3.3. C/N of *Porites astreoides* larvae following 20 h, 36 h, 60 h, and 80 h incubations in treatment water at 28°C in 2010. Dashed line represents baseline C/N, determined from samples taken at 0 h (i.e., immediately after release from parent colony and prior to introduction to treatment levels). Asterisks indicate significant differences (*). Sample sizes were as follows: N=10 at 0 h; N=4 (360 μatm), N=5 (471 μatm), N=5 (833 μatm) at 20 h; N=7 (360 μatm), N=6 (471 μatm), N=6 (833 μatm) at 36 h; N=5 (360 μatm), N=5 (471 μatm), N=5 (833 μatm) at 60 h; N=4 (360 μatm), N=3 (833 μatm) at 80 h (no larvae remained to be sampled from the 471 μatm treatment at 80 h).

Control respiration rates were approximately 2 nmol O₂ larva⁻¹ h⁻¹. If the respiratory quotient (RQ = CO₂ eliminated/O₂ consumed) is assumed to equal 1, the carbon specific respiration rate is ~ 0.024 μg carbon larva⁻¹ h⁻¹. Absolute amounts of carbon (μg
carbon larva\(^{-1}\)) were averaged for larvae that were collected immediately after release (40 ± 2 µg carbon larva\(^{-1}\), N=20) and for all larvae collected over all years/treatments (36.3 ± 0.8 µg carbon larva\(^{-1}\), N=174).

The respiration rates reported here (~2 nmol O\(_2\) planula\(^{-1}\) h\(^{-1}\) in controls) are lower than previously reported for this species and temperature (~5 nmol O\(_2\) planula\(^{-1}\) h\(^{-1}\) at 26°C, Edmunds et al. 2001). This is likely due to the longer dark-acclimation period in the present study. Coral larvae exhibit decreased respiration in the dark (*Pocillopora damicornis*, Gaither & Rowan 2010), and larvae in the present study were dark-acclimated for ~2 h prior to respiration measurements versus 10 min by Edmunds et al. (2001). Additionally, dark respiration rates were measured over the course of 2 h in this study, versus 20 min by Edmunds et al. (2001).

**Figure 3.4.** Larval metabolism as a function of \(p\text{CO}_2\). Data are pooled from 2 subsequent experiments. Closed triangles represent data collected 24 h after spawning; open triangles represent data collected 48 h after spawning. Error bars represent the analytical precision of the respiration rates. Respiration rates were measured using 20 larvae per treatment.
Table 3.3. Physical and chemical conditions at the start of respiration experiments (N = 1 initial). Incubations lasted for 2 hours and measured oxygen consumption of 20 larvae per treatment. All measurements are based upon duplicate or triplicate analyses for each sampling period. $p$H$_T$, $p$CO$_2$, HCO$_3^-$, CO$_3^{2-}$, CO$_2$, TCO$_2$, and $\Omega_{\text{arag}}$ were calculated using CO2SYS. Calcium concentration was calculated based on 10.28 mmol kg$^{-1}$ of Ca$^{2+}$ at a salinity of 35 ppt.
Settlement Experiments

Porites astreoides 2007

Percent settlement by treatment is presented in Table 3.4 for both May and June settlement experiments. Analysis of variance revealed that saturation state did not significantly affect the settlement rates of *P. astreoides* larvae; however, high within-treatment variance limited the statistical power to detect subtle treatment effects. Physical and chemical conditions during settlement experiments are provided in Table 3.5.

Table 3.4. Percent larval settlement of *Porites astreoides* in 2007 (Mean ± SEM). Settlement substrates were conditioned in ambient seawater; larvae were settled in treatment seawater (HCl-manipulated).

<table>
<thead>
<tr>
<th></th>
<th>Control Ω_a=3.2</th>
<th>Mid Ω_a=2.6</th>
<th>Low Ω_a=2.2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percent settlement</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>May</td>
<td>34.72 ± 7.29 (N=12)</td>
<td>41.94 ± 8.82 (N=12)</td>
<td>26.39 ± 5.83 (N=12)</td>
</tr>
<tr>
<td>June</td>
<td>12.50 ± 4.95 (N=8)</td>
<td>16.67 ± 4.88 (N=8)</td>
<td>13.33 ± 3.56 (N=8)</td>
</tr>
</tbody>
</table>

Porites astreoides 2008-2009

When settled onto Ambient Tiles, percent settlement declined by 11% at 560 µatm and 28% at 800 µatm, relative to controls. Percent settlement was as follows (mean ± SEM): 65 ± 8 (380 µatm); 58 ± 6 (560 µatm); and 47 ± 9 (800 µatm). Results of ANOVA indicate that these reductions in settlement are not statistically significant (F_{2,42}=2.07; P=0.14). When settled onto Treatment Tiles, percent settlement decreased by 43% at 560 µatm and 55% at 800 µatm relative to controls (F_{2,39} = 6.59, P<0.005) with percent settlement as follows: 65 ± 8 (380 µatm); 37 ± 10 (560 µatm); and 29 ± 4 (800 µatm) (Figure 3.5a). Results of post-hoc HSD Tukey analyses are presented in Table 3.6.
<table>
<thead>
<tr>
<th></th>
<th>Salinity</th>
<th>T (°C)</th>
<th>TA (µmol kg⁻¹)</th>
<th>pH</th>
<th>pCO₂ (µatm)</th>
<th>HCO₃⁻ (µmol kg⁻¹)</th>
<th>CO₃²⁻ (µmol kg⁻¹)</th>
<th>CO₂ (µmol kg⁻¹)</th>
<th>TCO₂ (µmol kg⁻¹)</th>
<th>Ca²⁺ (mmol kg⁻¹)</th>
<th>Ω₉arag</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>May</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>36 ± 1</td>
<td>26.6 ± 0.8</td>
<td>2348 ± 4</td>
<td>7.95 ± 0.02</td>
<td>513 ± 28</td>
<td>1863 ± 18</td>
<td>198 ± 7</td>
<td>13.6 ± 0.7</td>
<td>2074 ± 11</td>
<td>10.6 ± 0.3</td>
<td>3.1 ± 0.1</td>
</tr>
<tr>
<td>Mid CO₂</td>
<td>36 ± 1</td>
<td>26.6 ± 0.8</td>
<td>2206 ± 34</td>
<td>7.88 ± 0.02</td>
<td>575 ± 31</td>
<td>1799 ± 34</td>
<td>164 ± 6</td>
<td>15.3 ± 0.8</td>
<td>1978 ± 34</td>
<td>10.6 ± 0.3</td>
<td>2.61 ± 0.09</td>
</tr>
<tr>
<td>High CO₂</td>
<td>36 ± 1</td>
<td>26.6 ± 0.8</td>
<td>2138 ± 44</td>
<td>7.80 ± 0.02</td>
<td>701 ± 41</td>
<td>1801 ± 39</td>
<td>135 ± 7</td>
<td>19 ± 1</td>
<td>1955 ± 42</td>
<td>10.6 ± 0.3</td>
<td>2.2 ± 0.1</td>
</tr>
<tr>
<td><strong>June</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>34 ± 1</td>
<td>25.4 ± 0.3</td>
<td>2422 ± 14</td>
<td>7.99 ± 0.02</td>
<td>486 ± 23</td>
<td>1931 ± 14</td>
<td>202 ± 8</td>
<td>13.8 ± 0.7</td>
<td>2148 ± 11</td>
<td>10.0 ± 0.3</td>
<td>3.2 ± 0.1</td>
</tr>
<tr>
<td>Mid CO₂</td>
<td>35 ± 1</td>
<td>25.4 ± 0.3</td>
<td>2224 ± 55</td>
<td>7.91 ± 0.01</td>
<td>538 ± 32</td>
<td>1814 ± 62</td>
<td>166 ± 3</td>
<td>15 ± 1</td>
<td>1995 ± 62</td>
<td>10.3 ± 0.3</td>
<td>2.62 ± 0.04</td>
</tr>
<tr>
<td>High CO₂</td>
<td>35 ± 1</td>
<td>25.4 ± 0.3</td>
<td>2181 ± 9</td>
<td>7.84 ± 0.02</td>
<td>648 ± 37</td>
<td>1832 ± 14</td>
<td>141 ± 4</td>
<td>18 ± 1</td>
<td>1991 ± 12</td>
<td>10.3 ± 0.3</td>
<td>2.23 ± 0.08</td>
</tr>
</tbody>
</table>

**Table 3.5.** Physical and chemical conditions during *Porites astreoides* settlement experiments in 2007 (mean ± SD). Settlement substrates were conditioned in ambient seawater; larvae were settled in treatment seawater (HCl-manipulated). All measurements are based upon duplicate or triplicate analyses for each sampling period. $pH_T$, $pCO_2$, HCO$_3^-$, CO$_3^{2-}$, CO$_2$, TCO$_2$, and Ω$_{a_{HR}}$ were calculated using CO2SYS. Calcium concentration was calculated based on 10.28 mmol kg$^{-1}$ of Ca$^{2+}$ at a salinity of 35 ppt.
In 2009, when settled onto Treatment Tiles, percent settlement was reduced by 42% at 560 μatm and 60% at 800 μatm relative to controls (H=11.54, 2 d.f., P<0.005). Percent settlement was as follows: 53 ± 7 (380 μatm); 31 ± 6 (560 μatm); and 21 ± 5 (800 μatm). Results of settlement experiments with Treatment Tiles from 2008 and 2009 were pooled and analyzed via linear regression using least squares residuals, indicating a significant effect of pCO₂ on settlement success (F_{1,130}=29.58, P<0.0001) (Figure 3.5b).

Figure 3.5. Results of *Porites astreoides* settlement assays from 2008 and 2009 (mean ± SEM). (a) 2008: gray bars represent data from the Ambient Tile experiment (tiles conditioned in ambient seawater, larvae settled in treatment seawater); white bars represent data from the Treatment Tile experiment (tiles conditioned in treatment seawater, larvae settled in treatment seawater). (b) Pooled results from 2008 and 2009 Treatment Tile experiments. Open squares represent data from 2008. Closed squares represent data from 2009.
Table 3.6. Analysis of variance table and pair-wise multiple comparisons (Tukey’s HSD) of *Porites astreoides* Treatment Tile experiment in 2008.

<table>
<thead>
<tr>
<th>Settlement (Treatment Tile Experiment, 2008)</th>
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<tbody>
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<td></td>
<td>DF</td>
<td>SS</td>
<td>MS</td>
<td>F Ratio</td>
<td>P</td>
</tr>
<tr>
<td>Treatment</td>
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<td>1.370</td>
<td>0.685</td>
<td>6.594</td>
<td>0.0034</td>
</tr>
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<td>Residual</td>
<td>39</td>
<td>4.051</td>
<td>0.104</td>
<td></td>
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</tr>
<tr>
<td>Total</td>
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<tr>
<td>Tukey’s HSD</td>
<td>Mean Diff.</td>
<td>Q</td>
<td>(P&lt;0.05)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>342 v 537 µatm</td>
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<td>3.866</td>
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<td></td>
</tr>
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<td>342 v 765 µatm</td>
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<td>4.808</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>537 v 765 µatm</td>
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<td>0.8355</td>
<td>N</td>
<td></td>
<td></td>
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<tr>
<td>Salinity</td>
<td>T (°C)</td>
<td>TA (µ mol kg⁻¹)</td>
<td>pH₄</td>
<td>pCO₂ (µatm)</td>
<td>HCO₃⁻ (µ mol kg⁻¹)</td>
</tr>
<tr>
<td>---------</td>
<td>-------</td>
<td>----------------</td>
<td>-----</td>
<td>------------</td>
<td>------------------</td>
</tr>
<tr>
<td>Control (n=5)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>35.0 ± 0.4</td>
<td>28.4 ± 0.5</td>
<td>2359 ± 27</td>
<td>8.09 ± 0.04</td>
<td>339 ± 43</td>
<td>1704 ± 50</td>
</tr>
<tr>
<td>Mid CO₂ (n=8)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>34.9 ± 0.5</td>
<td>28.6 ± 0.6</td>
<td>2362 ± 21</td>
<td>7.94 ± 0.03</td>
<td>530 ± 48</td>
<td>1863 ± 32</td>
</tr>
<tr>
<td>High CO₂ (n=7)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>34.7 ± 0.7</td>
<td>28.8 ± 1</td>
<td>2362 ± 28</td>
<td>7.86 ± 0.09</td>
<td>678 ± 162</td>
<td>1928 ± 58</td>
</tr>
</tbody>
</table>

Table 3.7. Physical and chemical conditions during 2008 tile conditioning and settlement experiments with Porites astreoides.
Table 3.8. Physical and chemical conditions during Treatment Tile conditioning and settlement experiments with *P. astreoides* in 2009.
Acropora palmata 2009

Physical and chemical conditions during settlement experiments are provided in Table 3.10. Percent settlement was reduced by 45% at mid CO₂ and 69% at high CO₂ relative to controls ($F_{2,69}=21.02$, $P<0.0001$). Percent settlement was as follows: 63 ± 5 (491 µatm); 35 ± 5 (654 µatm); and 20 ± 4 (876 µatm).

![Figure 3.6](image)

Figure 3.6. Percent settlement of Acropora palmata by treatment (mean ± SEM).

<table>
<thead>
<tr>
<th>Settlement (Treatment Tile Experiment, 2009)</th>
<th>ANOVA</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>F Ratio</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>2</td>
<td>3.485</td>
<td>1.743</td>
<td>21.02</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>Residual</td>
<td>69</td>
<td>5.720</td>
<td>0.08290</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>71</td>
<td>9.205</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Tukey’s HSD</th>
<th>Mean Diff</th>
<th>Q</th>
<th>(P&lt;0.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td>491 v 654 µatm</td>
<td>0.3476</td>
<td>5.914</td>
<td>Y</td>
</tr>
<tr>
<td>491 v 876 µatm</td>
<td>0.5305</td>
<td>9.025</td>
<td>Y</td>
</tr>
<tr>
<td>654 v 876 µatm</td>
<td>0.1829</td>
<td>3.112</td>
<td>N</td>
</tr>
<tr>
<td></td>
<td>Salinity</td>
<td>T (°C)</td>
<td>TA (µ mol kg⁻¹)</td>
</tr>
<tr>
<td>----------</td>
<td>----------</td>
<td>--------</td>
<td>-----------------</td>
</tr>
<tr>
<td>Control</td>
<td>34.5 ± 0.1</td>
<td>28.0 ± 0.1</td>
<td>2339 ± 2</td>
</tr>
<tr>
<td>Mid CO₂</td>
<td>34.8 ± 0.1</td>
<td>28.0 ± 0.1</td>
<td>2388 ± 3</td>
</tr>
<tr>
<td>High CO₂</td>
<td>34.9 ± 0.1</td>
<td>28.0 ± 0.1</td>
<td>2336.2 ± 0.9</td>
</tr>
</tbody>
</table>

**Table 3.10.** Physical and chemical conditions during *Acropora palmata* settlement experiments in 2009. All measurements are based upon duplicate or triplicate analyses for each sampling period. $p_{H_T}$, $p_{CO_2}$, $HCO_3^-$, $CO_3^{2-}$, $CO_2$, $TCO_2$, and $\Omega_{arag}$ were calculated using CO2SYS. Calcium concentration was calculated based on 10.28 mmol kg⁻¹ of $Ca^{2+}$ at a salinity of 35 ppt.
**Substrate Community Composition Analyses**

**Point Count Analyses 2008**

Percent cover by CCA was reduced by 74% at 800 µatm, relative to the control ($t(18) = 6.998, P<0.0001$). Average percent cover of CCA was 42 ± 4 at 380 µatm and 11 ± 2 at 800 µatm (mean ± SEM).

![Figure 3.7](image)

**Figure 3.7.** Percent cover (mean ± SEM) of crustose coralline algae (CCA) on settlement tiles that were conditioned at 380 and 800 µatm over the course of 45 days in 2008.

**Spectrofluorometry Analyses**

*Porites astreoides 2009*

Epilithic algal communities of tiles that were pre-conditioned in ambient seawater (380 µatm) had significantly higher concentrations ($\mu$g gram$^{-1}$) of PE ($F_{2,28}=10.96, P<0.0005$) and PC ($F_{2,28}=18.38, P<0.0001$). PE concentrations were reduced by 78% and 74% at 560 and 800 µatm respectively, compared to controls, while PC concentrations were reduced by 73% and 83% (Figure 3.8a,b). Results of post-hoc HSD Tukey analyses
for PE/PC data are presented in Table 3.11. No significant differences were observed in the concentrations of chl \(a\) and chl \(c\) (Figure 3.8c,d). Chl \(b\) was not detected.

Absolute pigment concentrations (\(\mu g\) pigment gram\(^{-1}\) tile) by treatment were as follows (mean ± SEM): PE: 0.7 ± 0.1 (357 \(\mu\)atm); 0.15 ± 0.04 (555 \(\mu\)atm); 0.17 ± 0.04 (796 \(\mu\)atm). PC: 3.7 ± 0.6 (357 \(\mu\)atm); 1.0 ± 0.3 (555 \(\mu\)atm); 0.6 ± 0.1 (796 \(\mu\)atm). Chl \(a\): 1.1 ± 0.1 (357 \(\mu\)atm); 1.0 ± 0.1 (555 \(\mu\)atm); 1.00 ± 0.07 (796 \(\mu\)atm). Chl \(c\): 0.048 ± 0.007 (357 \(\mu\)atm); 0.056 ± 0.007 (555 \(\mu\)atm); 0.046 ± 0.004 (796 \(\mu\)atm).

**Figure 3.8.** Concentrations (mean ± SEM) of (a) Phycoerythrin (570 nm), (b) Phycocyanin (640 nm), (c) Chlorophyll \(a\) (670 nm) and (d) Chlorophyll \(c\) (635 nm) in biofilms of settlement tiles pre-conditioned at 26°C for 40 days (May, 2009) in treatment seawater (380, 560, 800 \(\mu\)atm). Concentrations were determined by measuring the fluorescent emission of the pigments extracted from the settlement tiles using a spectrofluorometer.
Acropora palmata 2009

At 40 d (time of settlement), epilithic algal communities of tiles that were pre-conditioned in elevated CO₂ (800 µatm) had significantly higher concentrations (µg gram⁻¹) of chl a (F₂,27=7.85, P<0.005) and chl c (F₂,27=6.90, P<0.005). Visual differences in tiles confirm these results, as tiles conditioned at elevated pCO₂ contained visibly more turf/fleshy algae, which appeared to impede larval settlement. Epilithic algal communities of tiles that were pre-conditioned in mid CO₂ seawater (555 µatm) had significantly higher concentrations (µg gram⁻¹) of PE (F₂,27=9.99, P<0.0001). However, no significant
differences in PC were observed and lack of visual differences raises the possibility that the measured differences were indicative of cyanobacteria instead of red algae.

Absolute pigment concentrations (µg pigment gram\(^{-1}\) tile) by treatment were as follows (mean ± SEM): PE: 0.10 ± 0.02 (380 µatm); 0.18 ± 0.03 (560 µatm); 0.06 ± 0.01 (800 µatm). PC: 0.26 ± 0.07 (380 µatm); 0.39 ± 0.07 (560 µatm); 0.27 ± 0.08 (800 µatm). Chl \(a\): 1.5 ± 0.1 (380 µatm); 1.5 ± 0.1 (560 µatm); 2.3 ± 0.2 (800 µatm). Chl \(c\): 0.065 ± 0.005 (380 µatm); 0.058 ± 0.009 (560 µatm); 0.11 ± 0.01 (800 µatm). Chl \(b\) was not detected.

**Figure 3.9.** Concentrations (mean ± SEM) of (a) Phycoerythrin (570 nm), (b) Phycocyanin (640 nm), (c) Chlorophyll \(a\) (670 nm) and (d) Chlorophyll \(c\) (635 nm) in biofilms of settlement tiles pre-conditioned at 28°C for 40 days (July, 2009) in treatment seawater (380, 560, 800 µatm). Concentrations were determined by measuring the fluorescent emission of the pigments extracted from the settlement tiles using a spectrofluorometer.
At 69 d, tiles conditioned at elevated CO$_2$ (800 µatm) still had significantly higher concentrations (µg gram$^{-1}$) of chl $a$ ($t$ (18)=3.058, $P<0.01$); however, differences in concentrations of Chl $c$ were no longer apparent. No significant differences were observed in PE concentrations between tiles; however, control tiles had significantly higher concentrations of PC than high CO$_2$ tiles (Kruskal-Wallis, $H=12.65$, 2 d.f., $P<0.005$). Absolute pigment concentrations (µg pigment gram$^{-1}$ tile) by treatment were as follows (mean ± SEM): PE: 0.31 ± 0.05 (380 µatm); 0.20 ± 0.04 (560 µatm); 0.21 ± 0.04 (800 µatm). PC: 0.9 ± 0.3 (380 µatm); 0.39 ± 0.06 (560 µatm); 0.23 ± 0.08 (800 µatm). Chl $a$: 2.6 ± 0.2 (380 µatm); 3.5 ± 0.2 (800 µatm). Chl $c$: 0.10 ± 0.04 (380 µatm); 0.09 ± 0.03 (800 µatm). No mid tiles were left at this point for chl analyses.

![Figure 3.10](image-url)

**Figure 3.10.** Concentrations (mean ± SEM) of (a) Phycoerythrin (570 nm), (b) Phycocyanin (640 nm), (c) Chlorophyll $a$ (670 nm) and (d) Chlorophyll $c$ (635 nm) in biofilms of settlement tiles pre-conditioned at 28$^\circ$C for 69 days (July-August, 2009) in treatment seawater (380, 560, 800 µatm).
Discussion

A significant effect of $pCO_2$ on larval settlement was only observed when tiles were conditioned in acidified seawater, with significant reductions in both 2008 and 2009, in both *Porites astreoides* and *Acropora palmata*. The results of both direct and indirect settlement experiments indicate that ocean acidification has the capacity to impact larval settlement but may primarily do so indirectly, by affecting the chemistry and microbiology of the substrata. The data suggest that, as CO$_2$ levels increase, changes in the algal community occur as red algae are outcompeted by other algal types, such as diatoms and other chromophytes. A decrease in the amount of phycoerythrin and phycocyanin (indicative of red algae) and an increase in the amount of chls $a$ and $c$ (indicative of diatoms and other chromophyta) are both changes that would serve to reduce the availability of settlement cues. These findings indicate that OA has the potential to alter coral recruitment dynamics by shifting epibenthic/epilithic algal community composition away from taxa known to facilitate larval settlement of certain coral species (e.g., CCA) and towards alternate algal species (e.g., consortia dominated by diatoms and other chromophytes). For further discussion of physiology and settlement experiments, refer to Chapter 5.
Chapter 4: Effect of Ocean Acidification on Post-Settlement Growth

Summary

While the effect of ocean acidification (OA) on the growth and calcification of adult reef corals is increasingly well documented, the effect on early, post-settlement growth is largely unknown. To investigate the effect of OA on post-metamorphic growth, lateral and/or linear extension was quantified for four Caribbean coral species, three brooders, *Porites astreoides, Favia fragum*, and *Agaricia agaricites*, and one broadcast-spawner, *Acropora palmata*. Growth was tested at three $pCO_2$ levels: ambient seawater (380 μatm) and two $pCO_2$ scenarios that are projected to occur by the middle (560 μatm) and end (800 μatm) of the century. In all species, post-settlement growth decreased with increasing $pCO_2$. Scanning electron microscopy of *P. astreoides* skeletons revealed that fine-scale skeletal morphology is altered as $pCO_2$ increases.

Background

Many benthic marine invertebrates, including corals, suffer high rates of mortality following settlement due to competition for limited resources such as light and space (Ritson-Williams et al. 2009). For corals, survival is positively correlated with juvenile growth rate and colony size (Hughes & Jackson 1985; Babcock 1991; Babcock & Mundy 1996) with up to a 20% increase in survivorship associated with a 0.5 mm increase in diameter of 4-month old juveniles of certain species (Babcock & Mundy 1996). Therefore, the faster a juvenile coral can grow, the more likely it is to survive and successfully recruit to the population.
Unfortunately, coral growth rates are expected to decline in coming years as atmospheric carbon dioxide levels increase and tropical surface waters become more acidic. Calcification rates of adult scleractinian corals and coralline algae have been shown to decline by 11-40%, measured over time periods ranging from 3 h to 2 years, with conditions mimicking a doubling of atmospheric carbon dioxide (Gattuso et al. 1998; Kleypas et al. 1999; Langdon et al. 2000; Leclercq et al. 2000; Marubini et al. 2001). While the response of adult scleractinians is increasingly well-documented, little is known regarding the sensitivity of juvenile growth rates to elevated CO$_2$.

To determine if and how near-future OA scenarios affect post-settlement growth of reef-building corals, juvenile growth was measured in 4 Caribbean coral species at 3 $p$CO$_2$ levels. Growth rates of three brooding species (*Porites astreoides*, *Agaricia agaricites*, *Favia fragum*) and one broadcast-spawning species (*Acropora palmata*) were analyzed from 2007-2009 using one of two methodologies. For *P. astreoides*, *A. agaricites* and *F. fragum*, lateral extension (i.e., increase in cross-sectional area, defined as the outermost extent of visible skeleton) was measured using low-magnification photographs. For *A. palmata*, linear extension was measured using an optical micrometer. Three $p$CO$_2$ levels were used to represent ambient seawater (380 µatm) and two $p$CO$_2$ increases that are expected to occur by the middle (560 µatm) and end (800 µatm) of this century (IPCC 2007). In 2007, carbonate chemistry was manipulated via HCl additions. In 2008-2009, carbonate chemistry was manipulated via direct bubbling with CO$_2$-enriched air (refer to Chapter 1 for details). Following growth experiments in 2007 and 2008, skeletons of *P. astreoides* were sampled for use in scanning electron microscopy.
(SEM) analyses to determine if and how elevated $p\text{CO}_2$ affects fine-scale skeletal morphology.

**Materials and Methods**

**Growth Experiments**

Porites astreoides 2007

Following settlement experiments in 2007, *P. astreoides* spat were introduced to treatment aquaria containing water corresponding to the treatment in which they were settled ($\Omega_{\text{arag}}=3.2$, 2.6, or 2.2). For methods concerning larval collection and settlement, please refer to Chapter 3. Duplicate aquaria were used for each treatment. Juvenile growth rates were determined by measuring the change in surface area over the course of 21 days in May and 28 days in June. Water temperature was maintained at $26.6^\circ\text{C} \pm 0.8^\circ\text{C}$ (mean ± SD) and $25.4^\circ\text{C} \pm 0.3^\circ\text{C}$ during May and June experiments, respectively. Ambient lighting was not artificially supplemented in order to discourage algal overgrowth of juvenile corals. Light intensity ranged from 1 to 191 $\mu$mol m$^{-2}$ s$^{-1}$, averaging less than 10 $\mu$mol m$^{-2}$ s$^{-1}$ over the course of 12.5 h of daily illumination. Juveniles were photographed under a dissecting microscope at the start and end of each experiment. SPOT© software was used to measure total surface area (defined as the outermost extent of visible skeleton). Growth rates were calculated as the rate of change in surface area (mm$^2$ month$^{-1}$). Data were square root transformed to meet assumptions of normality and analyzed using a one-way ANOVA. Details for water chemistry during the growth experiments are presented in Table 4.2.
Porites astreoides, Favia fragum, and Agaricia agaricites 2008

Following settlement experiments in 2008, *P. astreoides* juveniles on Ambient Tiles (refer to Chapter 3) were introduced to treatment aquaria containing water corresponding to the treatment in which they were settled. Individuals were mapped to allow for their identification over time, and growth (increase in cross-sectional area, defined as the outermost extent of visible skeleton) of each individual was quantified according to the methods outlined above. Growth rates (mm$^2$ month$^{-1}$) were calculated as the rate of change in cross-sectional area over time (49 days in May-June). Individuals exhibiting partial or full mortality were excluded from statistical analyses. Data were analyzed using a one-way ANOVA. D’Agostino & Pearson omnibus test and Levene’s test were used to verify the underlying assumptions of normality and homogeneity of variances, respectively. Where significant differences were detected, post-hoc HSD Tukey analyses were used to determine which treatments differed from each other.

In May 2008, coral colonies of *Agaricia agaricites* and *Favia fragum* were collected from Pickles Reef and Tavernier Rocks, respectively. All colonies were transported to the University of Miami’s Rosenstiel School of Marine and Atmospheric Science (RSMAS) and maintained in a flow-through seawater system for approximately two weeks during the predicted period of larval release. Larvae were collected according to the methods outlined by Kuffner et al. (2006). They were then settled onto limestone tiles that were pre-conditioned in ambient seawater for a minimum of two weeks, according to the methods outlined for *P. astreoides* in Chapter 3. Juvenile growth rates (lateral extension) were measured according to the previously outlined methodology over the course of fourteen days (June 15 – June 29, 2008). *A. agaricites* larvae settled on too
few tiles to measure growth in all $p$CO$_2$ treatments; therefore, only the two extreme $p$CO$_2$ levels (380 and 800 uatm) were used for this species. Growth for all three species (P. astreoides, A. agaricites, and F. fragum) was normalized to the controls; data were then pooled and analyzed via linear regression analysis.

Acropora palmata 2009

Following settlement experiments in 2009 (refer to Chapter 3), A. palmata juveniles were introduced to treatment aquaria containing water corresponding to the treatment in which they were settled and reared over the course of 50 d. Linear extension was routinely measured using an optical micrometer, capable of measuring linear extension rates to a precision of 0.5 µm. During each measurement period, juveniles were briefly removed from the treatment aquaria, fitted onto the stage of the micrometer, and advanced through the path of the optical micrometer. Each measurement was conducted in less than 3 min, thereby minimizing the time that spat were out of the water. Linear extension was calculated as the change in elevation over time. Individuals exhibiting partial or full mortality were excluded from the analysis. Data were analyzed by regression analysis.

Scanning Electron Microscopy

In 2007 and 2008, upon termination of the growth experiments, P. astreoides juveniles were sampled for use in scanning electron microscopy (SEM) to examine for potential effects of seawater pH/$p$CO$_2$ on fine-scale skeletal morphology. Juveniles were bleached in 10% sodium hypochlorite overnight to remove organic material. Skeletons
were rinsed three times with tap water and once with distilled water to remove bleach residue. Skeletal subsamples were mounted on aluminum stubs using carbon adhesive tabs and coated with palladium for three minutes using a Cressington 108 Auto sputter coater. Samples were imaged in an FEI XL-30 environmental scanning electron microscope (ESEM) to assess the presence of skeletal abnormalities at low pH (e.g., malformation or dissolution of the skeleton).

**Results**

**Growth Experiments**

Porites astreoides 2007

Saturation state exhibited a significant treatment effect on growth rates of *P. astreoides* juveniles in both May ($F_{2,178}=8.61, P<0.001$) and June ($F_{2,38}=10.46, P<0.001$). Absolute growth rates from both May and June experiments are provided in Table 4.1. Chemical conditions that persisted over the course of the experiments are provided in Table 4.2. Data from both months were pooled and analyzed by linear regression. Growth was positively correlated with saturation state ($P=0.007$) (Figure 4.1). Juveniles reared in the mid saturation state treatment grew an average of 45% (June) to 56% (May) slower than controls, while those reared in low saturation state treatments grew an average of 72% (May) to 84% (June) slower than controls.

Porites astreoides, Favia fragum, and Agaricia agaricites 2008

Chemical conditions during the course of the experiments are presented in Table 4.3. Growth rates for all three species declined with increasing $p$CO$_2$ (Figure 4.2).
Figure 4.1. Effect of saturation state on skeletal growth rate (mean ± SEM) of Porites astreoides juveniles in 2007 ($P = 0.003$).

Table 4.1. Juvenile growth rates (mm$^2$ month$^{-1}$) for Porites astreoides in 2007 (mean ± SEM (N), reared in controlled saturation state seawater (HCl-manipulated)).

<table>
<thead>
<tr>
<th>Growth rate</th>
<th>Control $\Omega_a=3.2$</th>
<th>Mid $\Omega_a=2.6$</th>
<th>Low $\Omega_a=2.2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>May</td>
<td>0.32 ± 0.03 (65)</td>
<td>0.14 ± 0.03 (79)</td>
<td>0.09 ± 0.05 (37)</td>
</tr>
<tr>
<td>June</td>
<td>0.44 ± 0.08 (14)</td>
<td>0.24 ± 0.03 (16)</td>
<td>0.07 ± 0.02 (10)</td>
</tr>
</tbody>
</table>

Absolute growth rates for all three species by treatment are presented in Table 4.4. Porites astreoides post-settlement growth significantly declined with increasing $p$CO$_2$ ($F_{2,99}=12.60$, $P<0.0001$). Results of post-hoc HSD Tukey analyses are presented in Table 4.5. Growth rates decreased by 16% and 35% at 548 and 775 µatm respectively, compared to controls. Favia fragum growth rates decreased by 21% at 537 µatm and 26% at 755 µatm compared to controls. Agaricia agaricites growth rates decreased by 27% at 755 compared to controls. Decreases in growth rates for both F. fragum and A. agaricites were not statistically significant, most likely due to low sample size and high within-treatment variance that limited the power to detect a treatment effect.
Table 4.2. Physical and chemical conditions during *Porites astreoides* growth experiments in 2007 (mean ± SD). All measurements are based upon duplicate or triplicate analyses for each sampling period. $pH_T$, $p$CO$_2$, HCO$_3^-$, CO$_3^{2-}$, CO$_2$, TCO$_2$, and $\Omega_{\text{arag}}$ were calculated using CO2SYS. Calcium concentration was calculated based on 10.28 mmol kg$^{-1}$ of Ca$^{2+}$ at a salinity of 35 ppt.
<table>
<thead>
<tr>
<th></th>
<th>Salinity</th>
<th>T</th>
<th>TA</th>
<th>pH_T</th>
<th>pCO₂</th>
<th>HCO₃⁻</th>
<th>CO₂⁺</th>
<th>CO₂</th>
<th>TCO₂</th>
<th>bCa²⁺</th>
<th>Ω_arag</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>36.0 ± 0.5</td>
<td>28.1 ± 0.2</td>
<td>2207 ± 36</td>
<td>8.08 ± 0.04</td>
<td>330 ± 35</td>
<td>1600 ± 39</td>
<td>244 ± 15</td>
<td>9 ± 1</td>
<td>1853 ± 35</td>
<td>10.6 ± 0.1</td>
<td>3.9 ± 0.2</td>
</tr>
<tr>
<td>Mid CO₂</td>
<td>35.9 ± 0.6</td>
<td>28.3 ± 0.2</td>
<td>2233 ± 45</td>
<td>7.91 ± 0.04</td>
<td>548 ± 55</td>
<td>1781 ± 47</td>
<td>183 ± 12</td>
<td>14 ± 1</td>
<td>1978 ± 45</td>
<td>10.5 ± 0.2</td>
<td>2.9 ± 0.2</td>
</tr>
<tr>
<td>High CO₂</td>
<td>36.1 ± 0.5</td>
<td>28.1 ± 0.2</td>
<td>2225 ± 46</td>
<td>7.78 ± 0.04</td>
<td>775 ± 98</td>
<td>1873 ± 61</td>
<td>142 ± 8</td>
<td>20 ± 3</td>
<td>2035 ± 58</td>
<td>10.6 ± 0.1</td>
<td>2.3 ± 0.1</td>
</tr>
</tbody>
</table>

Table 4.3. Physical and chemical conditions during 2008 juvenile growth experiments. All measurements are based upon duplicate or triplicate analyses for each sampling period. pH₇, pCO₂, HCO₃⁻, CO₂⁺, CO₂, TCO₂, and Ω_arag were calculated using CO2SYS. Calcium concentration was calculated based on 10.28 mmol kg⁻¹ of Ca²⁺ at a salinity of 35 ppt.
Figure 4.2. Juvenile growth rates (mean ± SEM) by pCO$_2$ treatment for (a,b) *Porites astreoides*, (c,d) *Favia fragum*, and (e,f) *Agaricia agaricites*. For *P. astreoides*, individuals exhibiting partial or full mortality were excluded from the analysis, resulting in fewer individuals in the mid and high pCO$_2$ treatments: N=38 (330 µatm); N=35 (548 µatm); N=29 (775 µatm). No mortality was observed of *F. fragum* or *A. agaricites* over the 14 d experiment.
Table 4.4. Growth rates (mm² month⁻¹) by treatment for three species of brooding corals, as measured over the course of 49, 14, and 14 d for Porites astreoides, Favia fragum, and Agaricia agaricites, respectively in May-June, 2008 (mean ± SEM).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Control (mean ± SEM)</th>
<th>Mid CO₂ (mean ± SEM)</th>
<th>High CO₂ (mean ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. astreoides</em></td>
<td>0.74 ± 0.04 (N=38)</td>
<td>0.62 ± 0.04 (N=35)</td>
<td>0.48 ± 0.03 (N=29)</td>
</tr>
<tr>
<td><em>F. fragum</em></td>
<td>1.9 ± 0.2 (N=12)</td>
<td>1.5 ± 0.1 (N=5)</td>
<td>1.4 ± 0.1 (N=13)</td>
</tr>
<tr>
<td><em>A. agaricites</em></td>
<td>3.57 ± 0.6 (N=17)</td>
<td>-</td>
<td>2.59 ± 0.4 (N=17)</td>
</tr>
</tbody>
</table>

Table 4.5 Analysis of variance table and pair-wise multiple comparisons of Porites astreoides growth experiment in 2008.

<table>
<thead>
<tr>
<th>ANOVA</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>F Ratio</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>2</td>
<td>1.0889</td>
<td>0.5445</td>
<td>12.6033</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Residual</td>
<td>99</td>
<td>4.2768</td>
<td>0.0432</td>
<td></td>
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<tr>
<td>Total</td>
<td>101</td>
<td>5.3657</td>
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<tr>
<th>Tukey’s HSD</th>
<th>Mean Diff.</th>
<th>Q</th>
<th>(P&lt;0.05)</th>
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<tr>
<td>330 v 548 μatm</td>
<td>0.1206</td>
<td>3.502</td>
<td>Y</td>
</tr>
<tr>
<td>330 v 775 μatm</td>
<td>0.2571</td>
<td>7.094</td>
<td>Y</td>
</tr>
<tr>
<td>548 v 775 μatm</td>
<td>0.1365</td>
<td>3.698</td>
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Figure 4.3. Relationship between juvenile growth rate (as a percent of the controls) and pCO₂ for three species of reef-building corals. Growth is indicative of lateral extension. Measurements were taken in 2008 in a CO₂ manipulated system.
There was a reduction in linear extension of 39% at the mid-CO$_2$ level and 50% at the high-CO$_2$ level compared to controls ($F_{1,23} = 4.86$, $P < 0.05$, linear regression analysis using least squares residuals) (Figure 4.4). The reduced sample sizes at the mid- and high-CO$_2$ levels resulted from greater partial and full mortality at these levels.

**Figure 4.4.** Linear extension (µm d$^{-1}$) of *Acropora palmata* juveniles by treatment over 50 d. Individuals exhibiting partial or full mortality were excluded from the analysis, resulting in fewer replicates in the mid- and high-CO$_2$ treatment groups (control, n=11; mid-CO$_2$, n=9; high-CO$_2$, n=5).
<table>
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<th></th>
<th>Salinity</th>
<th>T (°C)</th>
<th>TA (µ mol kg⁻¹)</th>
<th>pHf</th>
<th>pCO₂ (µatm)</th>
<th>HCO₃⁻ (µ mol kg⁻¹)</th>
<th>CO₃²⁻ (µ mol kg⁻¹)</th>
<th>CO₂ (µ mol kg⁻¹)</th>
<th>TCO₂ (µ mol kg⁻¹)</th>
<th>bCa²⁺ (mmol kg⁻¹)</th>
<th>Ω₉arag</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>34 ± 1</td>
<td>28.2 ± 0.1</td>
<td>2482 ± 42</td>
<td>8.04 ± 0.03</td>
<td>436 ± 41</td>
<td>1886 ± 53</td>
<td>247 ± 15</td>
<td>11 ± 1</td>
<td>2144 ± 46</td>
<td>10.0 ± 0.3</td>
<td>4.0 ± 0.2</td>
</tr>
<tr>
<td>Mid CO₂</td>
<td>34.2 ± 0.7</td>
<td>28.2 ± 0.1</td>
<td>2471 ± 32</td>
<td>7.93 ± 0.04</td>
<td>599 ± 69</td>
<td>1987 ± 55</td>
<td>200 ± 13</td>
<td>16 ± 2</td>
<td>2203 ± 46</td>
<td>10.0 ± 0.3</td>
<td>3.3 ± 0.2</td>
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<tr>
<td>High CO₂</td>
<td>35 ± 1</td>
<td>28.12 ± 0.09</td>
<td>2479 ± 37</td>
<td>7.79 ± 0.05</td>
<td>882 ± 111</td>
<td>2109 ± 52</td>
<td>154 ± 14</td>
<td>23 ± 3</td>
<td>2286 ± 46</td>
<td>10.1 ± 0.3</td>
<td>2.5 ± 0.2</td>
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</table>

**Table 4.6.** Physical and chemical conditions during *Acropora palmata* growth experiments in 2009 (mean ± SD; N=5 weekly samples). All measurements are based upon duplicate or triplicate analyses for each sampling period. pH_f, pCO₂, HCO₃⁻, CO₃²⁻, CO₂, TCO₂, and Ω₉arag were calculated using CO2SYS. Calcium concentration was calculated based on 10.28 mmol kg⁻¹ of Ca²⁺ at a salinity of 35 ppt.
Scanning Electron Microscopy

Quantitative reductions in juvenile growth rate with increasing $pCO_2$ are supported by SEM photographs, showing a loss of structural integrity in juvenile skeletons reared at elevated $pCO_2$. Whether this loss in integrity is due to malformation, dissolution, or a combination of the two is unknown. Qualitative differences in crystal morphology and organization were evident in $P. astreoides$ skeletons that were accreted in both HCl-manipulated seawater (2007) and CO$_2$-manipulated seawater (2008).

Skeletons of corals reared under ambient conditions consisted of long, acicular (i.e., needle-like) aragonite crystals that grew in a highly organized fashion, consisting of discretely packed bundles of well-aligned crystals that eventually coalesced to form smooth skeletal patches (Figure 4.5e). As $pCO_2$ increased and aragonite saturation state decreased, crystals became shorter and thicker (i.e., rhombohedron-like); a breakdown in organization seemed to occur such that crystals were oriented at random (i.e., not well-aligned); fewer crystal bundles formed and there was less coalescing (Figure 4.5f). Differences in crystal morphology were not quantified because it becomes increasingly difficult to obtain accurate measurements of crystal length as spat age, primarily due to the difficulty in identifying crystal bases as the skeletal structure increases in complexity (A. Cohen, *pers. comm.*). However, qualitative observations of changes in crystal morphology and orientation are consistent with Cohen & Holcomb (2009) and suggest that a decrease in crystal aspect ratio (i.e., ratio of crystal length to crystal width, *sensu* Cohen & Holcomb 2009) occurs as saturation state decreases.
Figure 4.5. Scanning electron micrographs of *Porites astreoides* spat reared at ambient ($\Omega_{\text{arag}}=3.2$ - panels a, c, e) and low ($\Omega_{\text{arag}}=2.2$ - panels b, d, f) aragonite saturation state (HCl-manipulated) over the course of 21 days in 2007. Scale bars for each view are indicated on SEM micrographs. (a,b) low-magnification view of entire spat; (c,d) experimentally-fractured septae; (e,f) high-magnification view of crystal morphology and orientation.
Figure 4.6. Scanning electron micrographs of *Porites astreoides* spat reared at ambient ($\Omega_{\text{arag}}=3.2$ - panels a, c, e) and low ($\Omega_{\text{arag}}=2.2$ - panels b, d, f) aragonite saturation state (HCl-manipulated) over the course of 21 days in 2007. Scale bars for each view are indicated on SEM micrographs. (a,b) low-magnification view of individual septae; (c-f) high-magnification view of crystal morphology and orientation.
Figure 4.7. Scanning electron micrographs of *Porites astreoides* spat reared at ambient CO$_2$ (330 µatm - left panels a, f, i), mid CO$_2$ (548 µatm - mid panels b, d, g) or high CO$_2$ (775 µatm - right panels c, e, h, j) (CO$_2$-manipulated) over the course of 49 days in 2008. Scale bars for each view are indicated on SEM micrographs. (a-c) low-magnification view of entire spat; (d,e) septae (f-h) close-up of individual septae; (i,j) high magnification view of crystal morphology and orientation.
Figure 4.8. Scanning electron micrographs of *Porites astreoides* spat reared at ambient CO$_2$ (330 µatm - left panels a, d), mid CO$_2$ (548 µatm - mid panel b) or high CO$_2$ (775 µatm - right panels c, e) (CO$_2$-manipulated) over the course of 49 days in 2008. Scale bars for each view are indicated on SEM micrographs. All images are high-magnification view of individual septa, showing changes in crystal morphology and orientation.

**Discussion**

Growth experiments conducted in all years (2007-2009) and all species (*A. palmata, P. astreoides, A. agaricites, F. fragum*) indicate that post-settlement growth rates, both lateral and linear extension, significantly decrease with increasing pCO$_2$. These findings are consistent with the hypothesis that saturation state controls calcification and, ultimately, growth, as has been documented for several adult scleractinians and an experimental reef community (Gattuso et al. 1998; Langdon et al. 2000, 2003; Leclercq et al. 2000, 2002; Marubini et al. 2001, 2002; Reynaud et al. 2003;
Langdon & Atkinson 2005; Fine & Tchernov 2007). Slowed post-settlement growth resulting from exposure to acidified conditions has been documented in a number of scleractinian coral species (Albright et al. 2008, 2010; Cohen et al. 2009; Suwa et al. 2010; Albright and Langdon 2011) and may translate into increased juvenile mortality since risk of mortality is inversely proportional to juvenile growth rate and colony size (Hughes & Jackson 1985; Babcock 1991; Babcock & Mundy 1996).

Quantitative reductions in juvenile growth rate with increasing $p\text{CO}_2$ are supported by SEM photographs, showing a loss of structural integrity in juvenile skeletons reared at elevated $p\text{CO}_2$. Whether this loss in integrity is due to malformation, dissolution, or a combination of the two is unknown. Qualitative differences in crystal morphology and organization were evident in $P. \text{astreoides}$ skeletons that were accreted in both HCl-manipulated seawater (2007) and CO$_2$-manipulated seawater (2008). For further discussion of growth experiments and SEM analyses, refer to Chapter 5.
Chapter 5: Effects of Ocean Acidification on Sexual Reproduction and Early Life History Stages of Caribbean Reef-Building Corals

The studies described in this dissertation demonstrate that near future ocean acidification (OA) scenarios have the potential to affect coral recruitment by impacting sexual recruitment and several early life history processes, including: sperm motility and fertilization success, larval metabolism, larval settlement, and post-settlement growth with implications for survival. Negative impacts on successive life history stages may cumulate in such a way that the overall effect on recruitment is devastating. For example, results of studies conducted with the threatened Caribbean elkhorn coral, *Acropora palmata*, indicate that OA has the potential to reduce fertilization success by 12-13% (averaged across all sperm concentrations) and to decrease settlement success by 45-69% at $pCO_2$ concentrations expected for the middle and end of this century. The compounding effect of OA on these early life history stages translates into a 52-73% reduction in the number of larval settlers on the reef. The net impact on recruitment will likely be even greater, given that depressed post-settlement growth is likely to result in elevated rates of post-settlement mortality. The implications of OA on each of the life history stages examined are discussed below.

**Fertilization**

Fertilization experiments, conducted with both *A. palmata* and *M. faveolata*, indicate that $pCO_2$ significantly affects fertilization rate. In both species, the effect of $pCO_2$ was dependent on the sperm concentration. At the highest sperm concentrations
(approaching optimal concentrations but unlikely to occur in nature), little to no treatment effect was observed; as sperm concentration declined, however, the effect of $pCO_2$ was exacerbated. No reduction in maximum potential fertilization was observed; however, higher sperm concentrations were necessary to achieve high fertilization success. In addition, comparison of slopes indicates a significantly higher fertilization rate in the control compared with either of the elevated CO$_2$ treatments. For $A.}$ *palmata*, the two elevated CO$_2$ treatments were not significantly different from each other. These results suggest that there may be a threshold response to elevated CO$_2$ within the range of CO$_2$ values and sperm concentrations that were tested for this coral species.

A recent study, conducted in sea urchins (*Strongylocentrotus franciscanus*), also showed a sperm-concentration-dependent effect of $pCO_2$ on fertilization success. In the study, Reuter et al. (2010) show that ocean acidification both decreases fertilization efficiency and increases susceptibility to polyspermy - fertilization by more than one sperm, resulting in unviable embryos. Results of Reuter et al. (2010), as well as those described here, underscore the need for caution when designing and interpreting experiments testing the effects of CO$_2$ on the fertilization of broadcast spawning marine invertebrates. The probability of detecting a treatment effect of $pCO_2$ depends on the sperm concentration used and how the fertilization curve changes under elevated $pCO_2$ (Reuter et al. 2010). It is therefore critical to utilize a large spectrum of sperm concentrations to fully understand the effects of acidification on marine invertebrate fertilization success. Sperm limitation (too few sperm to fertilize all eggs) is recognized as one of the key factors limiting reproductive success of sessile, broadcast-spawning marine invertebrates (Oliver & Babcock 1992; Levitan et al. 1992; Levitan & Peterson
1995). It is likely that in situ fertilization occurs at dilute sperm concentrations at which the effects of ocean acidification would be exacerbated. Results of both studies indicate that sperm concentrations that result in high fertilization success under present-day CO$_2$ concentrations may be considered sperm-limited in the future.

Decreased fertilization at increased $p$CO$_2$ may be due to physiological effects on the sperm, egg, or both. Decreased pH affects the intracellular pH ($p$H$_i$) in sperm, inhibiting mitochondrial respiration and motility (Christen et al. 1983). Suppressed sperm motility with decreasing pH has recently been demonstrated in at least three marine invertebrate species (urchins, Havenhand et al. 2008; sea cucumber, and coral, Morita et al. 2009; but see Havenhand & Schlegel 2009). While the sperm swimming speed data collected in 2009 for *A. palmata* and *M. faveolata* were inconclusive with respect to the effect of $p$CO$_2$, preliminary results from the IVOS system using *M. faveolata* sperm in 2010 indicate an effect of $p$CO$_2$ on sperm motility and swimming speed. Fertilization data from all years indicate that fertilization kinetics are being altered, and sperm motility is a likely candidate.

Egg susceptibility to fertilization may occur via multiple mechanisms, including interference with [$p$H$_i$] and/or increased susceptibility to polyspermy (Reuter et al. 2010). In sea urchins, the intracellular pH increases immediately following insemination (Lopo & Vacquier 1977), triggering the initiation of embryonic development (Johnson et al. 1976). The ability of molecular CO$_2$ to diffuse across cell membranes may decrease the [$p$H$_i$] such that the initiation of embryonic development is delayed or suppressed. Alternatively, an interference with block to polyspermy by CO$_2$ or hydrogen ions [$H^+$] would result in increased numbers of unviable eggs/embryos. As early as 1924,
researchers noted that sea urchin eggs (*Arbacia* spp.) fertilized in acidic conditions (pH 7.2) showed higher rates of polyspermy compared to pH 7.4-9.8 (Smith and Clowes, 1924). In 1932, Tyler and Schultz demonstrated that marine invertebrate fertilization is more susceptible to decreased pH than the later stages of development. Acidified seawater, pH 7.2, is sufficient to prevent fertilization of the marine worm *Urechis caupo*; however, if embryos are placed in acidic conditions after fertilization, they undergo normal cleavage and development at pH values as low as 6.4 (Tyler and Schultz, 1932). It is thought that the reason behind this observation is an interference with the initial stages of the fertilization reaction, namely, the block to polyspermy. Smith and Clowes (1924) concluded that this effect may be due to CO$_2$/H$^+$ interference with the formation of a fertilization membrane. Coral eggs do not have a fertilization membrane; however, Morita et al. (2006) suggest that coral eggs secrete motility suppressor(s) to prevent polyspermy. Thus acidification may interfere with the secretion of these putative motility suppressor(s), thereby inhibiting fertilization.

Effects of acidification on embryogenesis were documented over 25 years ago. Pagano et al. (1985) revealed irreversible teratogenic (‘dysmorphology’ or malformation) and genotoxic (deleterious effects on genetic material) effects from mild reductions in pH (-0.2 to -0.5 units using HCl additions) during sea urchin embryogenesis. Exposing embryos to acidified seawater during early stages of development resulted in increased mitotic abnormalities such as breaks, bridges, and lagging chromosomes and depression of mitotic activity (mitotoxicity). Developmental defects were also reported, including inhibited, malformed and/or blocked embryos. Exposure during later developmental
stages only resulted in the inhibition of skeletal differentiation, presumably as a result of decreased carbonate fixation.

More recently, elevated $pCO_2$ has been shown to negatively affect fertilization rate in some marine invertebrate species, but not others. For example, elevated $pCO_2$ negatively affects fertilization success in the oyster *Saccostrea glomerata* (Parker et al. 2009, 2010), and the urchins, *Echinometra mathaei*, *Hemicentrotus pulcherrimus* (Kurihara and Shirayama 2004; although significant effects were only observed when the acidification was severe: 5,000-10,000 ppm) and *Strongylocentrotus franciscanus* (Reuter et al. 2010). However, fertilization was unaffected by elevated $pCO_2$ in the oyster *Crassostrea gigas* (Havenhand & Schlegel 2009). Interestingly, multiple studies on a single urchin species, *Heliocidaris erythrogramma*, yield inconsistent results: Havenhand et al. (2008) showed that fertilization was heavily impacted by $pCO_2$ while Byrne et al. (2009, 2010) did not observe an effect.

It is likely that differences in results among studies are due to differences in methodology. As previously mentioned, sperm concentration can heavily impact experimental results (Albright et al. 2010; Reuter et al. 2010), and studies should employ a wide range of sperm concentrations to accurately assess the effect of acidification on fertilization. Only two of the aforementioned studies, Byrne et al. (2010) and Reuter et al. (2010), used more than one sperm concentration. In addition to sperm concentration, gamete compatibility can greatly impact fertilization success (M. Miller, I. Baums, *pers. comm.*; reviewed in Reuter et al. 2010). Therefore, studies employing a single male-female cross will likely yield different results from those using polyandry. Other factors that are known to influence fertilization success include: sperm–egg contact time, sperm
velocity and motility, egg size, gamete compatibility and sperm age (reviewed in Reuter et al. 2010).

Reproductive failure of corals due to sperm limitation is of increasing concern as hurricanes, disease, and other environmental and anthropogenic stressors continue to reduce coral population densities (i.e., Allee effects) (Levitan et al. 1992; Baums et al. 2005). This work demonstrates that ocean acidification threatens to diminish larval supply, a major determinant of recruit density (Hughes et al. 2000; Vermeij & Sandin 2008), and depressed recruitment will likely compromise coral reef resilience (the ability of reefs to recover after disturbance). Decreased larval supply may also hold implications for connectivity. Of the 428 coral species for which developmental mode has been described, 82.7% (354 species) are broadcast spawners and rely on external fertilization and planktonic development (Harrison 2011). Therefore, if A. palmata and M. faveolata are representative of spawning species in general, the fertilization and recruitment success of many spawning corals will likely be impaired as CO₂-driven ocean acidification intensifies.

**CHNS-O**

Consistent trends were observed in C/N by treatment in both 2008 and 2010. C/N was depressed at mid CO₂ levels relative to controls but was equal to or greater than controls at high CO₂. This persistent trend could be explained one of two ways: 1) changes in gene expression; and/or 2) concentration-dependent stimulation of photosynthesis at higher CO₂ values. Each of these potential mechanisms is addressed separately: 1) Prior work suggests that regulation of certain stress genes is dependent on
the magnitude of the stressor. For example, Edge et al. (2005) showed that, in response to temperature stress, expression of a urokinase plasminogen activator receptor (uPAR-like genes) in *Montastraea faveolata* increased at mildly elevated temperature but returned to background levels at the highest temperature (simultaneous with a down-regulation of ribosomal function). Similar patterns were observed in thioredoxin (TRX) expression in response to salinity stress (Edge et al. 2005). uPAR is found in several cell types and has multiple functions, including signal transduction, regulation of proteolysis, cytokine activity and cellular adhesion. Thioredoxin (TRX) is up-regulated in response to oxidative stress and hypoxia, amongst other environmental stressors and is also involved in cell proliferation, growth and development, and signal transduction. TRX may be a sensitive indicator of corals beginning to respond to changes in their environment (Edge et al. 2005). If moderate increases in CO$_2$ lead to increased levels of stress gene expression and the production of associated proteins, the increase in nitrogen could be reflected as a decrease in C/N. If gene expression is then suppressed at the highest CO$_2$ concentration (or returned to background levels), C/N would be restored to those of the controls. Recently, acidification has been shown to affect expression of a variety of genes in sea urchins (Todgham & Hofmann, 2009; O’Donnell 2010); similar work needs to be conducted in corals to better understand the mechanism behind changing larval condition. (2) A second, though not mutually exclusive, explanation for the observed trend in C/N ratios is a concentration-dependent stimulation of photosynthesis, where at high $p$CO$_2$ levels (e.g., 800 µatm), a critical CO$_2$ level is reached at which zooxanthellal photosynthesis is stimulated, increasing the production of photosynthate; this increase in photosynthate is reflected as an increased in C/N.
In 2009, following a 24 h incubation in treatment seawater, larvae in both the mid- and high-CO$_2$ treatments had significantly higher C/N than controls. The different trends observed in 2009 may be an artifact of temperature, as incubations were performed at 28°C in both 2008 and 2010 and at 26°C in 2009. To obtain more information from C/N data, future studies should: a) isolate CHNS-O analyses to recently spawned larvae to eliminate confounding factors from symbiotic algae (*Symbiodinium* spp.); b) sample at the same time of day to reduce/control for diurnal fluctuations in carbon and nitrogen; and c) employ a combined mechanistic approach (e.g., experimental and molecular) to better constrain the mechanism(s) responsible for the observed response.

**Metabolism**

Results of the respiration experiments demonstrate that near-future OA scenarios significantly depress larval metabolic rates. Metabolic suppression resulting from exposure to acidified conditions has previously been reported to occur in a variety of adult marine invertebrates, including: crabs (Metzger et al. 2007), squid (Rosa & Seibel 2008), worms (Pörtner et al. 1998), bivalves (adult and juveniles, Michaelidis et al. 2005), pteropods and amphipods (reviewed in Fabry et al. 2008). Recent work, conducted on sea urchin larvae, demonstrated that culturing larvae in acidified conditions resulted in the down-regulation of several genes involved in aerobic metabolism (Todgham & Hofmann 2009; O’Donnell et al. 2010). Metabolic suppression is considered an adaptive strategy for the survival of short-term hypercapnia and hypoxia (reviewed in Fabry et al. 2008); however, slowed metabolism is generally achieved by halting energy-expensive processes, such as protein synthesis (Hand 1991; Langenbuch et al. 2006), and therefore,
if sustained, will lead to reductions in growth and reproductive potential (Fabry et al. 2008). Thus, metabolic suppression is not considered to be advantageous under chronic elevations of CO$_2$, such as ocean acidification (Langenbuch & Pörtner 2004; Langenbuch et al. 2006).

Depressed metabolic rates in coral larvae may hold implications for larval fitness and motility, thereby limiting dispersal and settlement rates. Recent work demonstrated that oxygen consumption and energy use in *Acropora intermedia* peaks ~5 days after spawning, when larvae begin actively swimming and exploring (Okubo et al. 2008). During the planktonic dispersal phase, larvae actively explore and change their position in the water column to locate ideal settlement sites (Mundy & Babcock 1998; Raimondi & Morse 2000) and possibly influence horizontal transport and dispersal (Szmant & Meadows 2006). If metabolic suppression during the planktonic stage translates into decreased larval motility, the ability of larvae to regulate their vertical position in the water column may be compromised, thereby impacting dispersal and settlement potential. *Porites astreoides* is a brooding species, and the larvae contain symbiotic algae during the planktonic dispersal stage (as opposed to larvae of spawning species which generally do not contain symbiotic algae until after settlement/metamorphosis). These algae are likely providing the larvae with an additional source of energy in the form of translocated metabolites (Richmond 1982; Harrison & Wallace 1990), which may render them less susceptible to stressful environmental conditions. Recent work suggests that the nutritional status of a coral may play a role in its sensitivity to acidified conditions, with decreased sensitivity in individuals with supplemental food and/or nutrients (Cohen & Holcomb, 2009). Thus, larvae of broadcast-spawning species, devoid of symbionts, may
be more heavily impacted during the planktonic dispersal phase than symbiont-containing larvae of brooding species such as *P. astreoides*.

### Settlement

Settlement experiments, conducted over a 3-year period with both brooding (*Porites astreoides*) and spawning (*Acropora palmata*) coral species, investigated the effect of acidified seawater on larval settlement. In 2007 and 2008, the ability of OA to directly affect settlement (physiological disruption of settlement and/or metamorphosis) was assessed by conditioning tiles in ambient seawater and settling larvae onto those tiles in treatment seawater. Additionally, in 2008 and 2009, the potential for OA to indirectly affect larval settlement by altering the substrate community composition and the availability of biological and chemical settlement cues was assessed by conditioning tiles in treatment seawater and settling larvae in treatment seawater.

Results of the Direct Effect (Ambient Tile) experiments (2007 and 2008) indicate no significant effect of acidification on larval settlement. However, it should be noted that in 2007, low overall percent settlement coupled with high within-treatment variance meant that the power to detect subtle treatment effects was limited. In 2008, percent settlement decreased with increasing $\rho\text{CO}_2$. However, these results are not statistically significant, and it is not possible to determine whether the reductions in settlement are due to a non-significant effect of acidified water on larval physiology or whether the chemistry and microbiology of the settlement tiles were altered by acute pH shifts that occurred as tiles were moved to treatment water for the 24 h settlement experiment.
A significant effect of $p$CO$_2$ on larval settlement was only observed when tiles were conditioned in acidified seawater, with significant reductions in both 2008 and 2009, in both *P. astreoides* and *A. palmata*. The results of both direct and indirect settlement experiments indicate that ocean acidification has the capacity to impact larval settlement but may primarily do so indirectly, by affecting the chemistry and microbiology of the substrata.

Both positive settlement cues from crustose coralline algae (CCA) and settlement interference by turf algae have been previously documented (Morse et al. 1988; Webster et al. 2004; Birrell et al. 2005; Kuffner et al. 2006; Vermeij & Sandin 2008; Ritson-Williams et al. 2010). In 2008, point count data of settlement tiles conditioned at ambient (380 µatm) and elevated (800 µatm) $p$CO$_2$ showed a 74% reduction in percent cover of CCA, an effect which would presumably limit the availability of settlement cues. In 2009, spectrofluorometry data corroborated these differences in substrate community composition. Red or blue phycobiliproteins such as phycoerythrin (PE) and phycocyanin (PC) are major pigment characteristics of red algae (e.g., CCA) and/or cyanobacteria. Chlorophylls $a$ and $c$ are major pigment characteristics of chromophytes, such as Bacillariophyceae (diatoms), Dinophyceae (dinoflagellates) and Prymnesiophyceae (coccolithophores) (Rowan 1989; Jeffrey & Vesik 1997). In the 2009 *P. astreoides* experiments, biofilms that developed on tiles conditioned in ambient seawater contained significantly higher concentrations of phycoerythrin and phycocyanin. Differences in PE/PC concentrations cannot differentiate between red algae and cyanobacteria; however, visual differences in the tiles (noticeably more CCA present on tiles conditioned at ambient CO$_2$), indicated that the differences in PE/PC measured by spectrofluorometry
were indicative of CCA abundance as opposed to cyanobacteria. Therefore, the prevalence of phycoerythrin and phycocyanin on control settlement tiles may partially explain the higher settlement rates that were observed. These data are in agreement with previously published studies indicating that crustose coralline algae recruit and calcify more slowly at elevated CO₂ (Anthony et al. 2008; Kuffner et al. 2008).

In the 2009 A. palmata experiments, no significant differences were observed in the concentrations of PE and PC; however, there were significantly higher concentrations of chl a and chl c (indicative of diatoms) on tiles conditioned at high pCO₂ (800 µatm). Visible inspection of the settlement substrata confirmed that the higher pCO₂ treatments resulted in greater colonization by filamentous algae. The presence of these algae appeared to hamper larval settlement; upon scoring settlement, it was noted that larvae were exploring the substrate but not undergoing metamorphosis. Visible differences in colonization by CCA that were apparent approximately 3 wk after settlement experiments are consistent with results of the P. astreoides experiments and previously documented reductions in CCA colonization as a result of OA (Kuffner et al. 2008) and suggest that non-visible differences were present at the time of settlement. Both the increase in filamentous algae and the decrease in CCA colonization may explain the depressed settlement observed on tiles conditioned under elevated CO₂.

The data suggest that, as CO₂ levels increase, changes in the algal community occur as red algae are outcompeted by other algal types, such as diatoms and other chromophytes. A decrease in the amount of phycoerythrin and phycocyanin and an increase in the amount of chls a and c are both changes that would serve to reduce the availability of settlement cues. These findings indicate that OA has the potential to alter
coral recruitment dynamics by shifting epibenthic/epilithic algal community composition away from taxa known to facilitate larval settlement of certain coral species (e.g., CCA) and towards alternate algal species (e.g., consortia dominated by diatoms and other chromophytes).

Acidification has been shown to negatively impact larval settlement and/or metamorphosis in other marine invertebrates, including at least three species of marine bivalves (Talmage & Gobler 2009). Anlauf et al. (2011) reported no effect of acidified seawater on the ability of *Porites panamensis* larvae to successfully settle and metamorphose. However, this study tested only for direct effects of $pCO_2$ on larval settlement and did not address the potential for indirect effects by conditioning substrates in acidified seawater. Kurihara (2008) reported no effect of acidified seawater on the ability of *Acropora tenuis* larvae to successfully settle; however, materials and methods were not provided for these experiments, precluding direct comparisons with the results presented here.

**Post-Settlement Growth**

Growth experiments conducted in all years (2007-2009) and all species (*A. palmata, P. astreoides, A. agaricites, F. fragum*) indicate that post-settlement growth rates, both lateral and linear extension, significantly decrease with increasing $pCO_2$. These findings are consistent with the hypothesis that saturation state controls calcification and, ultimately, growth, as has been documented for several adult scleractinians and an experimental reef community (Gattuso et al. 1998; Langdon et al. 2000, 2003; Leclercq et al. 2000, 2002; Marubini et al. 2001, 2002; Reynaud et al. 2003;
Langdon and Atkinson 2005; Fine and Tchernov 2007). Larval and juvenile calcification may be more sensitive to acidification than adults, as has been shown for at least two marine invertebrates (one bivalve, one echinoderm; reviewed by Kurihara 2008). Maier et al. (2009) showed that calcification in younger polyps of *Lophelia pertusa* was more sensitive to acidified seawater than older polyps. Waldbusser et al. (2010) found a size dependent pH effect on calcification in juvenile hard clams (*Mercenaria* spp.), where smaller individuals were more heavily impacted than larger ones. For some marine invertebrates, the heightened sensitivity of early life history stages may, in part, be due to the presence of amorphous calcium carbonate (ACC) precursors that can occur at the onset of calcification and later stabilize into less soluble forms of CaCO₃. ACC is 30 times more soluble than calcite (Brecevic & Neilsen 1989; Politi et al. 2004), rendering it particularly vulnerable to acidified conditions. Many marine invertebrates undergo ontological changes in mineral composition, from ACC to aragonite and sometimes calcite. Larval spines of urchins form via an ACC precursor that later stabilizes into calcite (Beniash et al. 1997; Politi et al. 2008); the oyster, *Crassostrea virginica* secretes aragonite in the larval stages and calcite in the adult stage (Stenzel 1964); similarly, shell formation in molluscan larvae involves an initial, transient ACC phase (Weiss et al. 2002; Marxen et al. 2003), and it has been suggested that the same may be true for corals (Meibom et al. 2004, but see Clode et al. 2011).

Slowed post-settlement growth resulting from exposure to acidified conditions has been documented in a number of scleractinian coral species (Albright et al. 2008, 2010; Kurihara et al. 2008; Cohen et al. 2009; Suwa et al. 2010; Albright and Langdon 2011) and may translate into increased juvenile mortality. Risk of mortality has been
shown to be inversely proportional to juvenile growth rate and colony size (Hughes & Jackson 1985; Babcock 1991; Babcock & Mundy 1996) with up to a 20% increase in survivorship associated with a 0.5 mm increase in diameter of 4-month-old juveniles of certain species (Babcock & Mundy 1996). While post-settlement mortality was only observed in some, but not all, of the experiments reported here, it is important to note that mortality rates observed in this study do no approximate survivorship of juveniles in situ. Under laboratory conditions, factors known to affect early survivorship on the reef (e.g., competition with algae and other benthic organisms, sedimentation effects, predation) were controlled or eliminated in order to minimize influences on growth other than the desired treatment effect. Therefore, survivorship in this study likely overestimates survivorship that would be expected on the reef.

In addition to potential increases in juvenile mortality, both the onset of sexual maturity (Chornesky & Peters 1987; Szmant 1991) and fecundity (McGuire 1998; Babcock 1991; De Barros & Pires 2006) of reef-building corals are known to be a function of colony size. Therefore, depressed growth would likely result in longer time spent in juvenile (non-reproductive) life stages, which, in combination with adult loss, would shift population structures toward dominance by smaller size classes, ultimately reducing effective population sizes, population fecundity, and the resilience of reef-building corals (Done 1999).

**Conclusion**

Results of experiments presented here demonstrate that ocean acidification has the potential to accelerate the degradation of coral reefs by affecting sexual reproduction and
multiple early life history stages critical to reef persistence and resilience. These effects may occur via both direct (e.g., depressed sperm swimming speeds, fertilization, larval respiration, and growth) and indirect (e.g., changes in substrate conditions that favor settlement) pathways. Lowered fertilization limits larval supply, a major determinant of recruit density (Hughes et al. 2000; Vermeij & Sandin 2008), and depressed recruitment will likely compromise coral reef resilience (the ability of reefs to recover after disturbance). Slowed growth may trigger numerous repercussions, including, but not limited to elevated juvenile mortality and reduced recruitment success; and shifts in population size structure and lower reproductive output.

Sessile, broadcast-spawning organisms face several population bottlenecks during early life, including fertilization, settlement, and early post-settlement survivorship and growth (Vermeij & Sandin, 2008). As a result, natural larval and early juvenile mortality of many marine invertebrates often exceeds 99% (Gosselin & Qian 1997; Hunt & Scheibling 1997). For corals, recent research using artificial settlement substrates indicates that coral recruit survivorship during the first year is extremely low, generally reported to be as low as 0.2-6.0% survivorship, depending on the species and environment (Fairfull & Harriot 1999; Wilson & Harrison 2005). Stochastic events or chronic stressors that further reduce survivorship during these critical stages have the potential to significantly alter future population sizes (Vermeij & Sandin, 2008, Gosselin & Qian 1997). Although ocean acidification (OA) is now recognized as a substantial threat to marine calcifiers and their ability to secrete calcium carbonate shells and/or skeletons, the findings presented here demonstrate that increasing $p$CO$_2$ has the potential to impact multiple life history stages of corals, including critical processes independent of
calcification. The compounding nature of these impacts on successive life history stages suggests that the consequences of OA on coral populations and reef communities may be more severe than originally perceived. There is a need to further investigate the ability of corals to acclimatize and/or adapt to elevated $p\text{CO}_2$ given prolonged exposure, as well as the possibility of taxonomic differences in sensitivity. Future research should investigate the existence of genotypes and/or species that show resistance to pH/$p\text{CO}_2$ changes at multiple life history stages (e.g. fertilization, settlement, growth), as recruitment failure in these species may not be multiplicative. Focusing efforts on the protection and cultivation of more adaptable species or genotypes may improve the effectiveness of coral preservation and restoration efforts.
Works Cited


